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ACTION OF WHEAT AMYLASES ON RAW WHEAT STARCH [1]

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An investigation of factors governing rate of diastasis during prolonged auto-digestion of wheat flour, whether in water suspension or in dough, was reported recently by Sandstedt, Blish, Mecham, and Bode (1937). The data presented have justified the conclusion that in so far as diastatic susceptibility is concerned it is possible to distinguish between two main types of starch in wheat flour. By far the greater portion consists of the "raw," undamaged starch cells, highly resistant to diastasis. The lesser portion presumably includes the ruptured or damaged starch granules together with other starch-like material which may be more simply constituted than the "raw" starch portion. This lesser portion is relatively susceptible to diastatic saccharogenesis.

During the earlier stages of autolysis the more readily susceptible portion is rapidly hydrolysed to maltose by the flour amylase, and it is largely the rate and degree of this hydrolysis that are measured by the Rumsey method for determining "maltose values." Variations in "maltose values," as usually determined, are largely indicative of differences in the relative nature or amount of this so-called "susceptible" portion.

When the more readily available substrate has been used up, the rate of saccharogenesis diminishes rapidly and eventually tends to become a linear function of time. The rate of sugar production at this stage is presumably a measure of the rate at which the *raw* starch is being degraded. This rate of raw starch hydrolysis varies with different flours, and Sandstedt, Blish, Mecham, and Bode (1937) presented evidence strongly supporting a belief that these differences are

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due to variations in enzymic activity rather than to differences in the inherent susceptibilities of the raw starches themselves.

This is a report of more recent studies with special reference to the nature and properties of the enzymic and other biocatalytic factors specifically concerned with the hydrolytic degradation of raw wheat starch.

Basis for Present Classification of Amylases

There is, of course, no unanimity of opinion or belief as to the number, nature and identity of the enzymes, nor of certain accessory factors variously designated as activators, inhibitors, kinases, etc., that are involved in amylase activity. A fairly adequate summarization of the great mass of literature bearing upon the situation is to be found in a recent paper by Lüers (1936).

For present purposes it suffices to say that the leading amylase investigators are showing a definite tendency to recognize at least two distinct amylases, designated as alpha-amylase and beta-amylase. Beta-amylase, regarded as the chief amylase component of ungerminated grain, is primarily the saccharifying factor, while alpha-amylase, produced during germination, functions chiefly as a dextrinogenamylase.

It is highly important to note that the properties of these two amylases have been investigated, measured and defined largely, if not exclusively, in terms of their action, either singly or in combination, upon *artificially prepared starch; i.e.,* starch that has been rendered more or less " soluble " either by heating in water or by some other treatment. From the standpoint of comparative resistance to amylase, starch that has been rendered " soluble " by boiling or other artificial means is by no means comparable to the native " raw " or untreated starch. This is well exemplified in the case of potato starch, which, after boiling, is readily and almost completely saccharified by the combined action of alpha- and beta-amylase. In the raw state it is practically untouched by the combined action of the amylases of malted wheat flour.

The fact that soluble starch has been used as substrate almost exclusively in reported studies of amylase activity leaves very little basis for reliable judgment as to the identity, properties, and mode of action of the factor or combination of factors acting upon *raw, untreated* starch.

Occurrence of a " Raw Starch Amylase "

For obvious reasons, one expects to find the raw wheat starch amylase occurring largely, if not exclusively, in sprouted grain, as is actually the case. The same, of course, is true of alpha-amylase. This suggests that the two factors, whether or not identical, are likely to have similar properties. The work and the data herein reported are largely

some results of recent endeavors to show whether raw starch degradation is catalyzed exclusively by alpha-amylase or by some other unidentified biocatalytic factor or factors. It seems most unlikely that beta-amylase can be considered to play any part in initiating raw starch hydrolysis.

Extraction of the raw starch factor. It can readily be demonstrated that the raw "amylase" is extracted from flour by the same solvents that have been found suitable for extraction of alpha- and beta-amylase. Sandstedt, Blish, Mecham, and Bode (1937) showed that sodium chloride solution gives a much more complete extraction of flour amylase than does water alone, and consequently 5% salt solutions have been used habitually in these studies. If desired, these extracts can, without further treatment, be allowed to act directly upon raw wheat starch, the rate of conversion into maltose being observed at progressive time intervals.

The standard procedure in these studies has been to extract one part of flour with five parts of salt solution for about an hour, with occasional shaking. The extracts were removed with the centrifuge, saturated with toluol, and kept in the refrigerator. They can thus be kept for many weeks without undergoing significant change in enzyme activity.

Action of flour extracts on raw starch. When these flour extracts are permitted to act upon raw wheat starch, varying rates of maltose formation are readily observed. Figure 1 shows graphically the rates of maltose production when the extracts of several flours were added, respectively, to raw commercial wheat starch, and allowed to digest at 30° C., with toluol as a preservative. The procedure was to add 5 c.c. of extract to 10 g. of raw starch, with the addition of enough water (buffered to pH 4.7) to give a total volume of 100 c.c. Aliquots for maltose determination (by the ferricyanide reduction method) were withdrawn at successive time intervals.

In the case of the malted wheat flour extract, as shown in Figure 1, the rate of maltose conversion was many times greater than for the "sound" flours. The latter, however, showed very appreciable activities, as well as distinct differences in *rate* of sugar production.

In Figure 2 are shown the results of a similar study using the same flour extracts acting on raw starch, but in this series the enzyme extract and substrate were mixed together to form a paste containing 5 c.c. of extract, 5 g. of raw starch, and 0.3 g. of yeast. The rate of maltose conversion was observed by the yeast-manometric procedure, using the Sandstedt and Blish "pressuremeters" at 30° C.

It may be observed from Figure 2 that the yeast-manometric method gave essentially the same type of information as did the method involv-

ing the chemical estimation of reducing sugars at different time intervals. Both methods rated the flour extracts in precisely the same order as to their comparative activities on raw starch.

Pastes or doughs, such as were used in the yeast-manometric method, may reasonably be expected to afford a more homogeneous and therefore a more suitable medium than water suspensions in which the raw starch rapidly settles to the bottom of the flask unless constantly shaken.

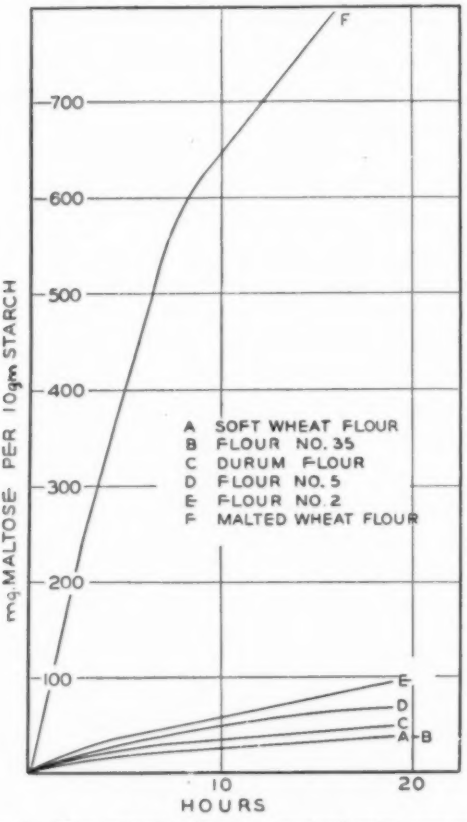

Figure 1. Flour extracts acting on raw starch-water suspensions.

To test this point, pastes were made just as for the yeast-manometric method, but no yeast was added, and rates of maltose production were determined, respectively, by withdrawing samples at appropriate time intervals, making to 100 c.c. with thorough shaking, and estimating maltose by the ferricyanide reduction method. The results of this series are shown in Figure 3.

The data represented by Figure 3 rate the flours in the same order as the data in Figure 1, but they show that the raw starch was hydro-

lysed considerably faster in the pastes than in the water suspensions, with the exception of the malted wheat flour extract. The exceptional behavior of the malted wheat flour may be due to its much greater enzyme concentration.

These observed differences in rates of activity between pastes and suspensions may appear to contradict the findings of Sandstedt (1934), who reported that rate of flour diastasis is essentially the same for suspensions as for doughs, other factors being equal. His work, however,

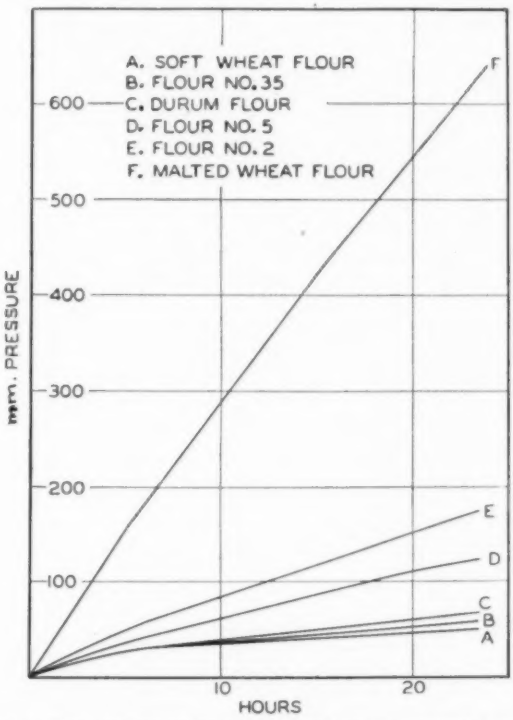

Figure 2. Flour extracts acting on raw starch—yeast manometric method.

dealt exclusively with *autolytic* digestions, in which the enzymes are much more intimately associated with the flour particles than is the case with extracts that are superimposed upon raw starch.

For sugar estimations by chemical methods the suspension method is more convenient than the "paste" method, because it permits the withdrawal of aliquots from the same digestion mixture at successive time intervals.

The yeast-manometric method is far the simplest and the most convenient of the three, and it has been found to be trustworthy and accurate.

Attention may now appropriately be directed to the fact that the flours used in the foregoing experiments are the same as were used in the work recently reported by Sandstedt, Blish, Mecham, and Bode (1937). In that work, involving comparative rates of *autolytic* conversion of starch into maltose, it was concluded that rate differences

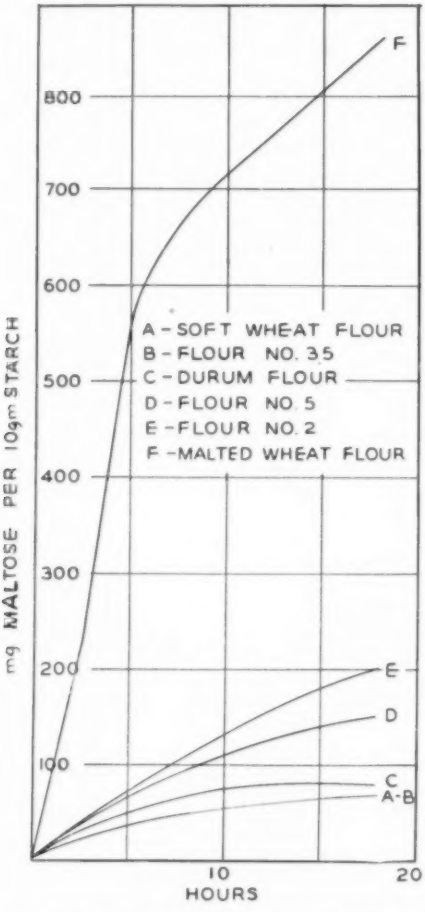

Figure 3. Flour extracts acting on raw starch-pastes.

during the later stages of auto-digestion were due to variations in amount of " raw starch amylase " among the different flours, not to inherent differences in starch susceptibility. The present data amply substantiate the former conclusions. The variations in rate of raw starch hydrolysis by the flour extracts in every instance, regardless of the method or modification used, consistently rank the flours in the same

order in which they were rated in the autolytic studies previously reported.

Identity of the Raw Starch Catalyst

It is now evident that flours from supposedly sound, ungerminated wheats contain, in varying amounts, appreciable quantities of a catalyst that is instrumental in converting raw wheat starch into maltose. Malted wheat flour, as expected, contains the factor in amounts much greater than ungerminated wheat flours. Since beta-amylase can reasonably be excluded as a factor of importance in initiating this phenomenon, and since some leading authorities consider that only beta-amylase, and not the alpha form, is present in ungerminated cereals, we are confronted at once with two possibilities: either the raw starch factor is separate and distinct from alpha-amylase, or, if alpha-amylase is indeed the factor involved, it must occur to an appreciable extent in *ungerminated* grain, despite authoritative opinion to the contrary.

In considering these alternatives it has seemed desirable to undertake to discover whether or not alpha-amylase and the " raw starch amylase " are identical or are separate and distinct factors. The first step in this undertaking has been an attempt to find a suitable means for obtaining the enzymes in concentrated form, reasonably free from extraneous matter and having stability over considerable time intervals.

The frequently used method of fractional precipitation from extracts with varying concentrations of ethyl alcohol was found objectionable because of progressive loss of activity when the enzymes stand for long periods in contact with alcohol. The rate of loss is definitely a function of temperature, as repeatedly cautioned by other workers. A quantitative study of this rate of loss in relation to temperature was reported recently by Blish, Sandstedt, and Mecham (1937).

After some use of the alcohol method it was discarded, at least temporarily, in favor of a technique involving precipitation of the amylases with ammonium sulfate, as originally used by Osborne (1895), and as later adapted for purification and fractionation studies by Sherman, Caldwell, and Doebbling (1934), and by Caldwell and Doebbling (1935). Our use of the method has differed from that of others essentially in having been applied to malted *wheat* instead of barley malt and in the use of *raw* wheat starch substrates as distinct from " soluble " potato starch, the latter apparently having been used exclusively by other workers.

Precipitation of malted wheat amylase with ammonium sulfate. When a flour extract is saturated, or nearly saturated with ammonium sulfate, the saccharogenic amylase (beta-amylase) is to all intents and purposes completely precipitated, and this strongly suggests that the

amylase is protein in character, as was pointed out by Osborne (1895). The precipitated amylase is separated either by filtration or with the centrifuge. It may be at once dispersed in water, and used in this form, or it may be dialyzed to remove the ammonium sulfate contamination.

One part of flour to five parts of 5% sodium chloride solution was used for extraction in these studies. When extracts of malted wheat flour and flour No. 2 were brought to 5/6 saturation with ammonium sulfate, and the precipitated amylases were allowed to act upon boiled wheat starch, the results were as typified in Table I.

TABLE I

ACTIVITIES OF ORIGINAL FLOUR EXTRACTS, AS COMPARED WITH AMMONIUM SULFATE PRECIPITATES, ON BOILED WHEAT STARCH

Enzyme preparation	Amount of flour extract represented	Substrate	Starch converted to maltose		
			1 hr.	17 hrs.	24 hrs.
	C.c.		%	%	%
Malted flour extract	2	1 g. starch[1]	65.0	73.5	76.5
Malted flour precipitate	2	1 g. starch	64.0	74.0	76.0
No. 2 flour extract	2	1 g. starch	48.5	59.5	61.0
No. 2 flour precipitate	2	1 g. starch	47.5	57.5	59.5

[1] 50 c.c. of 2% boiled wheat starch solution diluted to 100 c.c. after adding enzyme preparations and buffered to pH 4.7.

The data in Table I show that the activities of the ammonium sulfate precipitates on boiled starch were not significantly different from the activities of equivalent amounts of the original flour extracts.

When the enzyme extracts represented in Table I were allowed to act upon *raw* wheat starch, however, it was found that under comparable conditions the original extracts produced several times as much maltose as did the saturated ammonium sulfate precipitate. In one typical instance the original extract of malted wheat flour produced 766 mg. of maltose as against 122 mg. for the ammonium sulfate precipitate. There was considerable variation in the activities of these precipitates, but they were always much lower than the activity of the extract itself.

At first it seemed likely that all of the raw starch enzyme was not precipitated by the saturated ammonium sulfate, but no sugar-producing activity could be detected—either on raw or boiled starch—in the dialyzed supernatant liquid from the precipitations. It was then found that activity on raw wheat starch was greatly increased by considerably reducing the concentration of ammonium sulfate in the precipitation process. A number of precipitations were then carried out in which the

ammonium sulfate concentration was varied over a wide range. The precipitates were removed, dispersed in water and allowed to act on raw wheat starch. A typical series of these studies is shown in Table II. Ten cubic-centimeter portions of malted wheat flour extract were, respectively, brought to the desired ammonium sulfate concentration, and the precipitates removed with the centrifuge. Each precipitate was then washed into a small flask containing 5 g. of raw wheat starch, made to 50 c.c. and buffered to pH 4.7, digestion being carried out for 4 hours at 30°.

TABLE II

EFFECT OF VARYING CONCENTRATIONS OF AMMONIUM SULFATE ON THE RAW STARCH FACTOR OF THE PRECIPITATED AMYLASES

Ammonium sulfate	Maltose produced in 4 hours
%	Mg. per 10 gm. raw starch
None (original extract)	842
24	520
36	760
54	640
60	312
Saturated (70%)	278

It appears from Table II that half saturation with ammonium sulfate gives a precipitate that is far more active on raw starch than the one obtained by complete saturation. The 36% ammonium sulfate precipitate had not quite but almost as much activity as the original extract.

Activating and inhibiting biocatalysts. Since full saturation with ammonium sulfate would reasonably be expected to give more complete precipitation of the amylases than half saturation, some experiments were designed to afford some explanation of the foregoing unexpected results. The following tentative conclusions are the main outcome of these studies:

Germinated wheat contains, along with the raw starch enzyme, an inhibiting as well as activating substance. The "activator" is not precipitated by ammonium sulfate at any concentration. The inhibitor is increasingly precipitated with the amylase as saturation with ammonium sulfate is approached. At saturation, since the inhibitor is precipitated, while the activator is not present to counteract it, enzyme activity is greatly retarded. This was shown repeatedly by experiments of which the following is illustrative. In a malted wheat flour extract having an activity of 800, the precipitate obtained by 5/6 saturation with ammonium sulfate showed an activity of only 288. However, when the supernatant liquid from the ammonium sulfate precipitation, dialyzed

free from salt, was added to the precipitate, the activity was restored to 755, nearly equal to the original extract.

The "activator" is non-dialyzable; it is resistant to boiling-water temperature and hence non-enzymic. It is readily precipitated without loss of properties from solution by alcohol, acetone, etc. Used in almost any amount, within wide limits, it will counteract the effect of the inhibitor when the latter is present. It will not, by itself, act upon raw starch, nor does it appear to accelerate activity in the *absence* of the inhibitor.

The inhibitor, on the other hand, is apparently precipitated by full saturation with ammonium sulfate although not by half-saturation. It is dialyzable, as repeatedly shown by the fact that the low activity of saturated ammonium sulfate precipitates could be raised to normal merely by dialyzing the precipitate.

Dialysis, either of an ammonium sulfate precipitate or of the original extract itself, almost invariably causes a pronounced increase in the activity of the raw starch factor, other things being equal, due presumably to removal of the inhibiting substance.

Our present beliefs as to this phase of the situation may be summarized as follows:

1. Malted wheat flour contains a "raw starch amylase inhibitor," also a counteracting "activator."

2. When the activator is largely removed, leaving only the inhibitor, as in a saturated ammonium sulfate precipitate, raw starch hydrolysis is greatly retarded.

3. Although the activator is capable of counteracting the effect of the inhibitor, it does not substantially accelerate raw starch hydrolysis in the *absence* of the inhibitor. For this reason the term "activator" may be a misnomer. It is possible that the factor might be more appropriately designated as a "counter-inhibitor."

From these experiences it is clearly desirable, in using ammonium sulfate precipitation for the purpose of making raw starch amylase preparations free from inhibiting substances, that all precipitates be subjected to dialysis. Other advantages of this method of preparation are apparent. It of course greatly facilitates the concentration of the enzyme from large volumes of flour extract. The dialysis removes the considerable quantity of sugar produced by autolysis during the extraction of the flour, which would otherwise necessitate high "blank" corrections when studying rate of maltose production by the enzyme.

The fact that the saturated undialyzed ammonium sulfate precipitate showed the same activity on *boiled* starch as did the original extract itself (Table I), as contrasted with the comparative effects of the ex-

tract and the undialyzed saturated ammonium sulfate precipitate on *raw starch,* suggests that the raw starch amylase and alpha-amylase are not identical.

Sherman, Caldwell, and Doebbling (1934), and Caldwell and Doebbling (1935), using ammonium sulfate precipitation for their studies of the *soluble* starch saccharifying amylases of barley malt, dialyzed their preparations for purposes of purification. Their values, reported in terms of activity *per unit weight of enzyme substance,* not in terms of the original malt, show that the dialysis was highly effective in eliminating foreign material. Whether or not their treatment removed substances that were actually inhibitory, as in the case of the " raw starch amylase," is not clear.

Enzyme preparations made from wheat flour by this procedure, whether dialyzed or not, may be saturated with toluol and kept for weeks in the refrigerator without significant loss of orginal activity.

Fractional precipitation of raw starch amylase with ammonium sulfate. It was deemed advisable to study in some detail the extent to which the malted wheat flour amylases are precipitated by varying concentrations of ammonium sulfate. This work covered the range from 10% up to complete saturation (about 70%), and each fraction was removed before the succeeding fraction was precipitated.

As a starting point, 600 g. of malted wheat flour were extracted with 3000 c.c. of 5% NaCl solution. By centrifuging, 2750 c.c. of extract were recovered, 5 c.c. being equivalent to 1 g. of flour. A 100 c.c. portion of the extract was dialyzed, and its activity compared with that of the original extract. Another portion was saturated at once with ammonium sulfate, and the precipitate was dialyzed. Another portion was made to 35% ammonium sulfate, and the precipitate was dialyzed. The supernatant liquid from this precipitation was made on up to 70% ammonium sulfate, and the additional precipitate dialyzed.

For the detailed fractional precipitation study 1000 c.c. of the extract were made to an ammonium sulfate concentration of 10%. The precipitate was removed by centrifuge, and the remaining liquid was made to 15%. The additional precipitate was removed and the process repeated progressively, with the result that precipitates were obtained for concentration ranges, respectively, of 0 to 10%, 10 to 15%, 15 to 20%, 20 to 25%, 25 to 30%, 30 to 40%, and 40 to 50% of ammonium sulfate. Each precipitate was individually re-dispersed in water, and re-precipitated from the same concentration of ammonium sulfate originally used. After re-precipitation each preparation was dialyzed, made to a definite volume, treated with toluol and stored in the refrigerator.

Dialysis was in running tap water, and during dialysis considerable material separated out of dispersion, the amount of this precipitated material being progressively less in the fractions from the higher concentrations of ammonium sulfate. No attempt was made to filter out this deposited material, but each preparation was shaken thoroughly to a uniform suspension when an aliquot was withdrawn for further work.

Each preparation was studied with reference to its activity on each of three different substrates. The substrates selected were soluble (boiled) potato starch, raw commercial wheat starch,[2] and a preparation of "erythrogranulose" made according to the method specified by Hanes (1935).

Erythrogranulose was selected in the hope that the activities of the various preparations on this substance, as compared with corresponding activities on raw wheat starch, might furnish a trustworthy indication as to whether the raw starch factor is or is not perhaps identical with alpha-amylase.

This presumption is based upon the following considerations: Erythrogranulose is prepared by allowing beta-amylase, alone, to act upon soluble starch until the limit of maltose conversion is reached, which as Hanes (1935) has shown represents about a 60% conversion to maltose. The remaining material, designated as erythrogranulose, is precipitated with alcohol and dried with strong alcohol and ether. Erythrogranulose is highly resistant to further maltose conversion by beta-amylase but it is susceptible to alpha-amylase, the rate and degree of its conversion to maltose depending upon the quantity of alpha-amylase added, as shown by Hanes (1935). He also demonstrated that alpha- and beta-amylase in combination will produce more maltose from erythrogranulose than will alpha-amylase alone, despite the fact that beta-amylase, alone, is without effect. From these considerations it seems probable that if indeed alpha-amylase and the raw starch amylase are one and the same thing, the various fractions obtained from malted wheat flour should show comparative potentialities for attaching raw wheat starch that are in the same order as their activities on erythrogranulose.

In studying rates of maltose conversion with the different substrates, the aliquot of the enzyme preparation used in each case represented 2 g. of the original malted wheat flour. With the soluble (boiled) potato starch substrate this aliquot was added to 50 c.c. of 1% starch solution, which was then buffered to pH 4.7 and made to 100 c.c. With the erythrogranulose a 1% solution of erythrogranulose solution was substituted for the potato starch, other things being the same. With the

[2] Central Scientific Co.

raw wheat starch substrate 5 g. of starch were shaken with 100 c.c. of buffered solution containing the enzyme.

In all cases, the solutions or mixtures were saturated with toluol and allowed to digest at room temperature, aliquots being withdrawn at various intervals for maltose estimation by the ferricyanide procedure of Blish and Sandstedt (1933). Controlled temperature was not used, since comparative rather than absolute values were desired. In the case of the raw starch series, it was necessary to shake the flasks at occasional intervals, as the starch rapidly settles to the bottom.

Comparative rates of maltose conversion in the different series are shown in Tables III, IV and V, and in these tables the various enzyme preparations are designated as follows:

X—Original flour extract.
DX—Original flour extract dialyzed.
0–10—Precipitated at 10% concentration of ammonium sulfate.
10–15—Precipitated in the 10 to 15% concentration range.
15–20—Precipitated in the 15 to 20% concentration range.
20–25—Precipitated in the 20 to 25% concentration range.
25–30—Precipitated in the 25 to 30% concentration range.
30–40—Precipitated in the 30 to 40% concentration range.
40–50—Precipitated in the 40 to 50% concentration range.
0–70—Saturated ammonium sulfate precipitate.
0–35—35% ammonium sulfate precipitate.
35–70—Precipitated in the 35 to 70% concentration range.

TABLE III

ACTIVITIES OF VARIOUS AMYLASE FRACTIONS ON SOLUBLE (BOILED) POTATO STARCH

Enzyme preparation	Reducing sugars calculated as maltose				
	15 min.	4 hrs.	25 hrs.	48 hrs.	168 hrs.
	G. per 10 g. of starch				
X	7.92	8.84	10.68	11.12 [1]	11.90 [1]
DX	8.44	9.08	10.44	10.94	11.90 [1]
0–10	2.78	6.64	7.92	8.26	8.84
10–15	1.94	7.14	7.88	8.34	8.98
15–20	4.28	8.00	8.78	9.36	9.70
20–25	7.48	8.44	9.42	9.80	9.92
25–30	5.28	7.76	8.34	8.50	8.78
30–40	3.60	7.48	8.06	8.22	8.22
40–50	.18	1.54	5.84	6.56	7.62
0–70	8.22	8.84	10.20	10.72	11.22
0–35	8.26	8.84	10.04	10.48	11.06 [1]
35–70	.34	2.56	7.32	7.68	8.06

[1] These quantities are higher than can be accounted for on the basis of the starch used. This is interpreted to mean that there was some conversion of maltose to glucose on long standing, and since glucose has a higher reducing action per unit weight than maltose, the too high values may thus be explained.

TABLE IV

ACTIVITIES OF VARIOUS AMYLASE FRACTIONS ON RAW WHEAT STARCH

Enzyme preparation	Reducing sugar calculated as maltose			
	13 hrs.	24 hrs.	52 hrs.	168 hrs.
	G. per 10 g. of starch			
X	.71	1.99	2.48	3.49
DX	.72	2.31	3.02	4.76
0–10	.05	.18	.31	.53
10–15	.07	.26	.39	.67
15–20	.31	.71	.91	1.38
20–25	.40	1.39	1.68	2.48
25–30	.23	.53	.70	1.04
30–40	.13	.38	.50	.75
40–50	.02	.02	.04	.07
0–70	.58	2.00	2.48	3.67
0–35	.56	1.90	2.29	3.28
35–70	.02	.05	.09	.14

TABLE V

ACTIVITIES OF VARIOUS AMYLASE FRACTIONS ON ERYTHROGRANULOSE

Enzyme preparation	Reducing sugar calculated as maltose			
	4 hrs.	24 hrs.	48 hrs.	76 hrs.
	G. per 10 g. of erythrogranulose			
X	4.84	7.18	7.86	8.40
DX	5.10	7.00	7.52	8.02
0–10	1.42	2.76	3.26	3.90
10–15	1.90	3.00	3.60	4.12
15–20	3.16	4.42	4.76	5.16
20–25	4.26	5.50	5.96	6.26
25–30	3.30	3.68	3.98	4.12
30–40	2.48	3.26	3.66	3.78
40–50	.44	1.02	1.30	1.60
0–70	4.96	6.42	7.10	7.54
0–35	4.86	6.32	7.00	7.44
35–70	.98	2.18	2.58	3.00

Activities of enzyme preparations on boiled starch. The data in Tables III, IV, and V show not only the comparative maltose producing activities of the different enzyme preparations, but they also clearly point out distinctive differences as to the manner in which the three different substrates are attacked by the *same* enzyme preparation.

Activity on the soluble (boiled) starch is extremely rapid in the initial stages of digestion, only 15 minutes being sufficient to convert more than 2/3 of the total starch into maltose in certain cases. Following this initial stage, the rate of saccharogenesis greatly diminishes until after a few hours it is almost negligible. This is typical of the manner in which beta-amylase attacks soluble starch, as has been shown repeatedly by others, including Caldwell and Doebbling (1935) and Hanes

(1935). Hanes (1935) considers that with beta-amylase, alone, activity stops when about 60% of the total soluble starch has been converted to maltose, but when alpha-amylase is also present maltose production can go far beyond 60% starch conversion. If this viewpoint is correct, it is apparent that all of the preparations dealt with in Table III contained both alpha and beta-amylase (since more than 60% of the starch was converted in all cases), and that ammonium sulfate fractionation did not afford means for separating the two.

The method used with soluble starch is, of course, the Lintner type of procedure. Employing such a procedure with highly active enzyme preparations it is obvious that one must either use a very short time interval, or an exceedingly small quantity of enzyme in order to measure differences in enzyme activity. Thus in 15 minutes (Table III) enzyme fraction 10–15 showed a maltose-producing value of 1.94, only 36.7% as high as that of preparation 25–30, which had a value of 5.28. After 4 hours, however, the value of the former was 92% of the latter, the figures being respectively, 7.14 and 7.76.

Activities of the various enzyme preparations on raw starch. An entirely different type of activity is shown by the various enzyme preparations on raw wheat starch. Under the experimental conditions represented in Table IV, activity starts comparatively slowly, but progresses at a fairly uniform rate. Here it is found that differences in potentiality for saccharifying raw wheat starch are shown to advantage only after *long* periods of digestion, in distinct contrast to the situation where *soluble* starch is the substrate. Thus, for example, X (the original extract) and DX (dialyzed extract) show nearly equal values after 13 hours, but from that point DX steadily forges ahead of X until at 168 hours X (value 3.49) has converted only 73% as much starch as DX (value 4.76).

The raw starch values shown in Table IV were, of course, obtained by the water-suspension method, chiefly on account of convenience in permitting the progressive withdrawal of aliquots from a single sample. From considerations previously discussed in connection with the values presented in Figures 1, 2, and 3, however, activities of the various enzyme preparations on *raw starch* may have progressed at a much higher rate if *pastes,* instead of suspensions, had been used.

Activities of the various enzyme preparations on erythrogranulose. With erythrogranulose as the substrate, the action of the amylases was in most cases of a type that is somewhat intermediate with reference to the activities on the soluble and raw starches, respectively. Initial rate was rapid, as in the case of the soluble starch, but differentiation could not always be made until after several hours. Over a long period

of time, however, the various preparations tended to maintain the differences established after the first few hours of diastasis and in the final stages did not approach each other as closely as did the soluble starch values shown in Table III.

The final values (76 hours) are presumably indicative of differences in alpha-amylase activity, for reasons previously discussed.

Figures 4 and 5 give typical rate curves for the purpose of illustrating graphically the issues presented in the foregoing discussion. Summarizing the essential features of this phase of the discussion, the curves show that (1) differences in activity upon soluble starch registered very prominently in the initial stage of diastasis (15 minutes) but not at 168

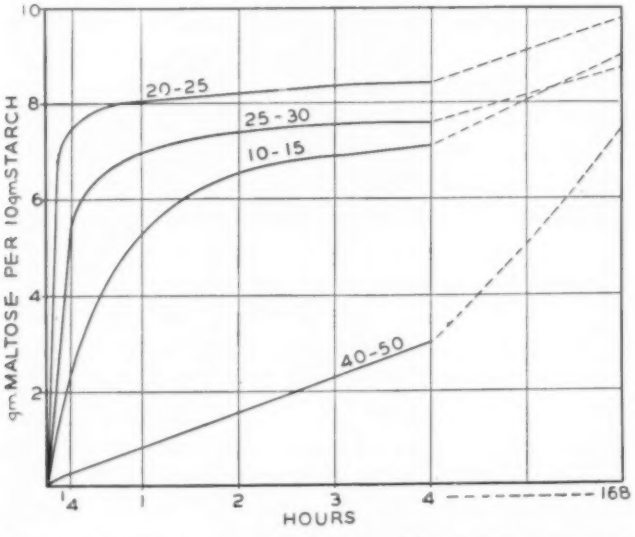

Figure 4. Action of enzyme preparations on soluble (boiled) potato starch.

hours; (2) differences in *raw starch* hydrolysis, conversely, become more and more apparent as diastasis progresses; whereas (3) with erythrogranulose as substrate differences in most instances register at all stages of diastasis, although it is advisable to allow several hours to elapse before distinct differences are well established.

Comparative activities of the various enzyme fractions. Fractionation with ammonium sulfate showed that a 35–40% concentration of this salt is sufficient to precipitate practically all of the amylases from an extract of malted wheat flour. The raw starch factor, in particular, is completely precipitated at 35% ammonium sulfate concentration (see fractions 35–70 and 40–50), and is present only in very small amount in concentrations of less than 15%. Of the various individual am-

monium sulfate fractions, 20–25 showed the greatest activity on all three substrates, which probably indicates that all of the amylases are most highly concentrated in that fraction.

The original extract (X) did not differ greatly from the dialyzed extract (DX) in its action either on soluble starch or erythrogranulose. Upon the raw starch substrate, however, the dialyzed extract was very significantly more active than the original undialyzed extract.

Is raw wheat starch "amylase" identical with alpha-amylase? The fact that dialysis, either of the original malted wheat flour extract or of the precipitate obtained by saturation with ammonium sulfate, greatly increases activity on raw wheat starch but does not significantly alter the activity either on soluble starch or on erythrogranulose, suggests

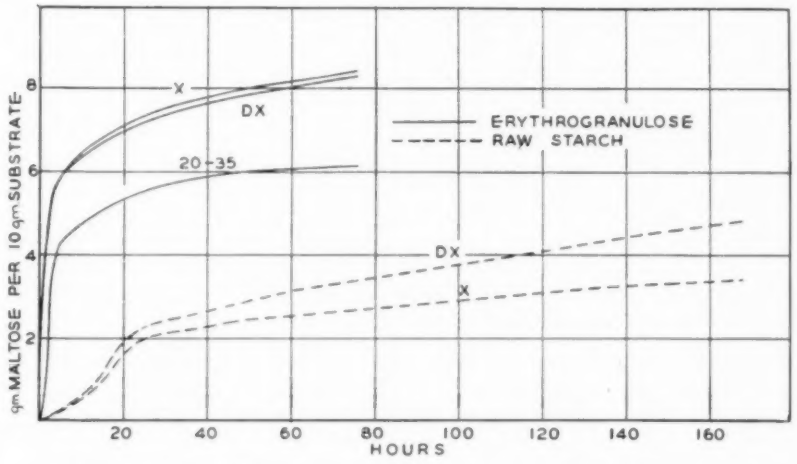

Figure 5. Action of enzyme preparations on raw starch and on erythrogranulose.

that the enzyme hydrolysis of raw wheat starch is not attributable to alpha-amylase, but that some other catalyst or combination of factors must play a fundamental part in the process.

This is also suggested by comparing the activities of certain of the ammonium sulfate fractions on erythrogranulose and on raw starch, respectively. In Tables IV and V, fractions 0–10, 10–15, and 25–30 all showed, in 76 hours, the same degree of activity on erythrogranulose, the values being, respectively, 3.90, 4.12, and 4.12. On raw starch, however, fractions 0–10 and 10–15 gave values on raw starch of 0.53 and 0.67, respectively, while fraction 25–30 gave 1.04, or about twice the values of 0–10 and 10–15. This suggests that alpha-amylase is more precipitable at the lower concentrations of ammonium sulfate than is the raw starch amylase. The range of precipitation with ammonium

sulfate (salt concentration) appears to be somewhat wider for alpha-amylase than for raw starch amylase.

The foregoing conclusions are, of course, based upon the assumption that activity on erythrogranulose is, in fact, a reliable index to the quantity of alpha-amylase present, an assumption which seems justified in the light of Hanes' (1935) work. It was deemed advisable, however, to confirm these findings, if possible, by the use of other methods for estimating alpha-amylase.

One of the oldest and most commonly used is the method of Wohlgemuth (1908), whereby the rate of disappearance of the blue color with iodine, after the enzyme preparation has been added to soluble starch, is considered indicative of the amount of alpha-amylase present. The method has several disadvantages, but is considered reasonably reliable when used under strictly comparable conditions. In this investigation the conditions were adjusted to show the amount of amylase (in terms of weight of original flour) required to cause complete disappearance of the blue iodine color in 6 hours. There is doubtless a considerable factor of error, especially with the most active preparations.

Table VI shows the comparative values on some of the preparations that are represented in Tables III, IV, and V.

TABLE VI

ALPHA-AMYLASE VALUES AS ESTIMATED BY A MODIFIED WOHLGEMUTH PROCEDURE

Preparation	Amount required to produce decoloration in six hours
	Grams of original flour
X	0.04
DX	0.05
0–10	2.4
10–15	2.3
15–20	0.6
20–25	0.18
25–30	0.4
30–40	1.4
40–50	20.0 plus

The results given in Table VI confirm, for the most part, those of Table V, showing activities on erythrogranulose, and they rate the preparations in essentially the same order. With respect to preparation 25–30, however, the two methods fail to agree, for reasons that are not apparent to us.

The alpha-amylase activities of X and DX are again shown to be the same, whereas their comparative activities on raw starch are significantly different, again suggesting that the two biocatalysts involved are different.

Additional confirmatory evidence on this point is furnished by the fact that the Wohlgemuth method showed the soft wheat flour to be entirely lacking in alpha-amylase, whereas it consistently showed appreciable saccharifying power on raw starch.

A third method, believed to afford an especially trustworthy and convenient measure of alpha-amylase activity, is an alcohol precipitation method, based upon a procedure reported by Caldwell and Hildebrand (1935), who proposed as a suitable measure of amylase activity the rate at which a given enzyme preparation causes soluble potato starch to lose its property of precipitating with ethyl alcohol. It is believed that this method can afford an exclusive and reliable measure of *alpha-amylase* activity, especially if used on a solution of erythrogranulose instead of soluble starch as the substrate. Erythrogranulose, having been already exhaustively acted upon by beta-amylase, is no longer susceptible to its further action, unless alpha-amylase is also present. Therefore, if an excess of beta-amylase is added, together with the unknown amylase preparation, the beta-amylase factor should be entirely eliminated as an influencing variable, and the results, under controlled conditions, should reflect differences in alpha-amylase exclusively.

Fifty cubic centimeter portions of a 1% solution of erythrogranulose, buffered to pH 4.7, were used. To each portion was added 1 c.c. of the preparation to be tested, and 1 c.c. of 5% NaCl extract of soft wheat flour (1 part of flour to 5 of solution) was also added to supply an excess of beta-amylase. At the expiration of 10 minutes the mixture was poured into 100 c.c. of ethyl alcohol. After standing over night the precipitated starch was filtered on a sintered glass crucible, washed finally with strong alcohol and ether, dried in a vacuum oven, and weighed.

Typical results with a few of the amylase fractions are shown in Table VII.

TABLE VII

RESULTS WITH ALCOHOL PRECIPITATION METHOD FOR ESTIMATION OF ALPHA-AMYLASE ACTIVITY

Preparation	Starch not precipitated after 10 minutes' activity
	%
X [1]	70.5 [1]
DX [1]	68.8 [1]
10–15	7.1
15–20	31.1
20–25	71.6
25–30	34.4

[1] Only 1/5 as much enzyme material was used as with the other four preparations. If compared on an equal basis, X and DX would have been more active than 20–25.

It is seen from Table VII that the four ammonium sulfate "fractions" fall in the same order, with respect to alpha-amylase activity, as they did by the Wohlgemuth method (Table VI), fraction 20–25 always showing the greatest activity.

Here again the alpha-amylase activities of the dialyzed (DX) and undialyzed (X) extracts are substantially the same, in contrast to their different rates of activity on raw starch.

The ammonium sulfate fractionation studies of Caldwell and Doebbling (1935) seem to show that with barley malt the alpha-amylase portion is very largely precipitated at the lower concentrations (up to 20%) of the salt. The data in Tables V, VI and VII do not show that this is correspondingly true of the amylases of malted wheat flour.

Precipitation of raw starch amylase by alcohol. Van Klinkenberg's (1932) belief that alpha-amylase is precipitated from malt extract by adding ethyl alcohol to 60% by volume, whereas beta-amylase is thrown down only by an alcoholic concentration of 80% by volume, has been widely accepted. It therefore seemed appropriate to apply alcohol fractionation in studying the properties of the *raw* wheat starch amylase.

Ten cubic centimeter aliquots of a 5% NaCl extract of malted wheat flour (1 part of flour to 5 parts of solvent) were made, respectively, to 50%, 60%, 70%, 80%, 90% and 93% of alcohol by volume, and held at ice-water temperature for an hour. The precipitates were removed by centrifuge, re-dispersed in water and allowed to digest with raw wheat starch for 4 hours at 30° C. Under these conditions the amounts of starch converted to maltose were as shown in Table VIII.

TABLE VIII

ACTIVITIES OF RAW STARCH AMYLASE PREPARATIONS PRECIPITATED FROM VARIOUS ALCOHOL CONCENTRATIONS

Alcohol by volume	Maltose produced in 4 hours
%	Mg. per 10 g. of starch
50	190
60	560
70	830
80	860
90	830
93	840

The data in Table VIII appear to show that although some of the raw starch amylase is precipitated at an alcoholic concentration as low as 50% by volume, approximately 70% is necessary for complete precipitation. A more complete study of the properties of the alcohol precipitates should, however, be made before conclusions may be safely drawn from this phase of the investigation.

Optimum hydrogen-ion concentration for raw starch amylase activity. Ten cubic centimeter portions of the malted wheat flour extract were added, respectively, to several 5 g. portions of raw wheat starch, each one buffered to a different pH, the range covered being pH 3.5 to pH 7.10. Reducing sugars in suitable aliquots were determined after 24 hours' digestion, with results as shown in Figure 6.

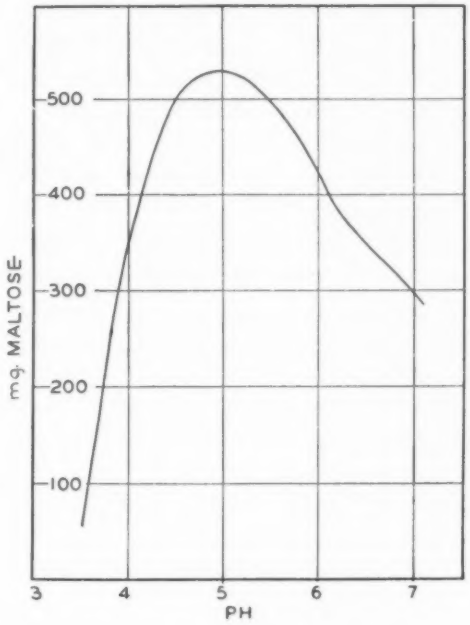

Figure 6. Effect of pH on activity of raw starch amylase.

The data in Figure 6 indicate pH 5 as the optimum reaction for the amylase that is active on raw wheat starch.

Amylase Preparations from "Sound" (Ungerminated) Flours

The methods of preparation, fractionation and study of the amylases of malted wheat flour have been applied also to ordinary commercial flours, some of which are believed to contain no germinated wheat material. Such flours, as compared with malted flour, to be sure, contain only small, though variable quantities of raw starch amylase. However, we have found none that are entirely lacking in this factor. Soft wheat flours contain the least of any that we have studied.

In applying various possible methods for concentrating and fractionating the amylases of normal flours, the results have been entirely

consistent with what would be anticipated from experiences with the malted wheat flour.

Perhaps one of the simplest methods for preparing the flour amylases in concentrated form is to extract with salt solution, dialyze the extract until free from salt, and then concentrate the dialyzed solution by "pervaporation," which consists merely of exposing the dialyzing bag with its contents to a current of air from an electric fan, whereby the water in the bag is rapidly evaporated without heat. By this means a large volume of dialyzed extract can be concentrated to almost any reasonable degree without loss or injury to the properties of the enzymes. On the other hand, if desired, the enzymes may be first precipitated with ammonium sulfate, and the precipitate may then be dialyzed and pervaporated. Numerous possible modifications of these types of procedure are at once apparent.

Regardless of the method of preparation, we have invariably found that, under comparable conditions, the activities of these preparations on raw starch always rate the flours in the same order as they would fall if studied by the autolytic procedure of Sandstedt, Blish, Mecham, and Bode (1937), who concluded that in the later stages of autodigestion, when a slow, though uniform rate of saccharogenesis is reached, the different rates observed with different flours are indicative of corresponding variations in the quantity of "raw starch amylase" contained in the flours. This conclusion is abundantly substantiated by these later findings, as illustrated by Figures 1, 2 and 3.

Using various dialyzed amylase preparations, with raw wheat starch as substrate, the yeast-manometric procedure is highly convenient, accurate and reliable, just as it proved to be for the autolytic studies. The yeast method is, however, not suitable for preparations containing considerable amounts of salts, nor for solutions containing toluol.

Miscellaneous Observations

Throughout the course of these studies occasional phenomena were observed, some of which were unexplainable while others appeared conspicuously incompatible with existing viewpoints that seem to have become fairly well established. For instance, an extract of presumably sound barley showed appreciable alpha-amylase activity when tested by the Wohlgemuth method (disappearance of iodine color), although ungerminated barley is generally reported to contain no alpha-amylase. On the other hand, soft wheat flour extracts showed no alpha-amylase by the Wohlgemuth test, but when extracts of the two were, respectively, allowed to act upon soluble potato starch, under comparable conditions,

the soft wheat extract gave a smaller yield of "erythrogranulose" than did the barley extract.

There seems to be a widespread belief that raw starch owes its resistance to amylases largely to a more or less impervious outer covering of a cellulose-like material, and that when once this covering is ruptured, or otherwise removed, the inner portion ("amylose") then becomes readily available to saccharification. Our observations do not appear to support this viewpoint, but tend rather to indicate a more homogeneous structure of the starch granule.

This conclusion is drawn from observing the steady and uniform rate at which raw starch is saccharified over a long period of time. Preparation DX (see Table IV and Figure 5) converted more than 40% of the raw starch to maltose in 168 hours. Nevertheless, during this time there occurred no abrupt change in rate indicating that an impervious outer coating had been removed, exposing a more readily available inner portion.

Summary and Conclusions

Normal wheat flour contains an enzyme factor capable of accelerating the saccharification of raw, untreated wheat starch. The amount of this factor varies with different flours, soft wheat flour probably containing the least.

These variations in quantity of raw starch amylase explain why, during *autolytic flour digestions* over long periods of time, especially in the later stages and after the readily susceptible portion of the starch has been saccharified, different flours digest their own *raw* starch at different rates.

The "raw starch amylase" can readily be extracted from flours, with dilute sodium chloride solution. The extracts, when allowed to act, respectively, upon portions of the *same* raw starch, will hydrolyze the starch at different rates, and these different rates are found to fall into the same order as the rates observed in the autolytic digestions.

Amylase preparations containing the raw starch catalyst can readily be prepared in concentrated form by precipitation with ammonium sulfate and dialysis of the precipitate, followed, if desired, by "pervaporation" of the dialyzed extract; or they may be prepared merely by dialysis of the original flour extract, followed by pervaporation. The most highly active preparations are, of course, those from malted wheat flour. These preparations are stable, and can be kept for long periods of time without significant loss of activity.

The raw starch amylase is almost completely precipitated from malted wheat flour extracts by ammonium sulfate over a range of from

15 to 35% concentration of the salt. Fractionation studies showed it to be most highly concentrated in the range of 20 to 25% ammonium sulfate.

A non-enzymic inhibitor, also a non-enzymic " activator " of the raw starch factor, have seemingly been identified in malted wheat flour extract. The former is dialyzable and is precipitated by saturated ammonium sulfate. The latter is not dialyzable and is not precipitated by ammonium sulfate.

The raw starch amylase shows properties that appear to distinguish it definitely from alpha-amylase.

The yeast-manometric method is a most convenient and trustworthy procedure for studying the activity of flour amylase extracts acting upon raw starch as a substrate, just as it has been found eminently suitable for *autolytic* flour amylase studies.

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CORRELATION OF EXPERIMENTAL AND COMMERCIAL BAKING TESTS WHEN USING SPONGE DOUGHS. II [1]

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(Read at the Annual Meeting, June 1936)

The general aims and purposes of this work were discussed in a previous communication on correlation between bakery and laboratory results for straight doughs,[3] the primary objective of which communication was to determine whether it is possible to produce in the laboratory a loaf similar to one produced in a commercial bakery, and if so, the variations of laboratory conditions that would accomplish such a result. The second objective was to obtain from laboratory results information in regard to the kind of bread which will be produced in the bakery when a given laboratory formula and procedure are used.

In studying sponge doughs, methods similar to those for straight doughs were used covering the different steps involved in the sponge-dough procedure.

Formula and Procedure

A typical, commercial type formula was used:

Sponge	%	Dough	%
Flour	60	Flour	40
Water	34	Water	32
Yeast	1.8	Sugar	5
Malt extract	0.5	Salt	2
Yeast food [Arkady]	0.3	Shortening [lard]	3
		Malt extract	0.5
		Skimmilk powder	6

Material

The flour used in the sponge stage was a northwestern patent, and that in the dough stage a Kansas patent.

Methods

The procedure in the bakery was a follows: The sponge ingredients were mixed for 3 minutes, the sponge temperature after mixing being

[1] Subcommittee report, 1935–36 Committee on the Standardization of Laboratory Baking.

[2] The authors wish to acknowledge the valuable assistance of Stanley McHugh.

[3] Freilich, Jacob, McHugh, Stanley, and Frey, Charles N. Cereal Chem. 12: 668 (1935).

76° F. The sponge was placed in a dough room maintained at 80° F., and fermented for 4½ hours, during which period the sponge temperature changed from 76° to 89° F. The sponge was then placed in the mixer with the dough ingredients, and the dough mixed for 10 minutes, with the dough temperature 80° F. at the end of mixing. The dough was permitted to stand 15 minutes, then put through the divider and rounder, and after 14 minutes' bench proof, was molded and panned, then proofed at 95° F. and baked in an electrically heated oven for 35 minutes, with steam in the oven during the first few minutes of baking.

The same formula and ingredients were used in the laboratory, with variations in procedure for the individual or combined steps as indicated below.

Sponge Mixing

Experiments were made in which a wide range of mixing time was used in the laboratory mixer when mixing sponges. Bakery-mixed sponges were used as controls. After the bakery sponge was mixed, a small part of it was immediately taken to the laboratory and divided into a number of smaller portions, each equal in weight to that of the individual laboratory sponges. After mixing, both bakery and laboratory sponges were handled in exactly the same way in the laboratory, so that mixing was the only variable.

The results of two sponge-mixing experiments are shown in Table I.

TABLE I
VARIATIONS IN SPONGE MIXING TIME

	Sponge mixing time	Sponge fermentation time	Dough time	Pan proof	Volume per pound of bread
	Minutes	*Hours*	*Minutes*	*Minutes*	*C.c.*
		Experiment 1			
Bakery sponge	3	5	30	46	2380 [1]
Laboratory sponge	1	5	30	46	2400
	2	"	"	45	2340
	4	"	"	47	2340
	6	"	"	44	2400
	8	"	"	47	2300
	10	"	"	47	2330
	12	"	"	47	2310
	14	"	"	47	2340
		Experiment 2			
Bakery sponge	3	5	30	46	2380 [1]
Laboratory sponge	1	5	30	48	2400
	2	"	"	45	2400
	3	"	"	44	2380
	4	"	"	46	2360
	6	"	"	47	2430
	8	"	"	48	2400

[1] Average of 3 loaves.

The results of both experiments shown in Table I were very similar with respect to loaf volume and bread characteristics. In exterior appearance and crumb color the loaves made from the bakery and laboratory sponges were about alike, with the exception of the laboratory sponges mixed 10, 12 and 14 minutes, which produced loaves slightly whiter than the others in crumb color. The differences in texture and grain were of no apparent significance, with the exception of the laboratory sponges mixed 12 and 14 minutes, in which cases the grain was more open. The volume differences were all within experimental error.

It may therefore be said that there is a wide range of sponge-mixing time—from 1 to 10 minutes for the Fleischmann mixer—within which satisfactory bread, equal to that made from bakery-mixed sponges, may be produced by using a laboratory mixer.

It should be noted that the sponges mixed for a long time were quite different in character from those mixed for a short time, but this made no apparent differences in the resulting bread.

Comparative Farinograph curves for bakery and laboratory sponges are shown in Figure 1.

Sponge Fermentation

In the sponge fermentation experiments, only the bakery-mixed sponge was used. Immediately after the sponge came out of the bakery mixer, a small part of it was divided into laboratory size portions which were fermented for varying lengths of time under laboratory conditions at 80° and 86° F. After sponge fermentation, portions of the large bakery sponge were taken to the laboratory and subsequently handled in exactly the same way as those sponges which had been fermented under laboratory conditions.

The figures shown in Table II were obtained in two experiments in which the laboratory size sponges were fermented for varying lengths of time at 80° F. in air.

The temperature of the small sponges, which had been taken from the large sponge mixed at 76° F., increased with time, till a maximum of 83½° F. was reached after 4½ hours, following which there was a drop to about 82½° F. in the 6½- and 7½-hour sponges.

The loaves resulting from experiment 1, Table II, showed significant differences.

In crust color, the 1½-hour sponge produced the darkest loaf and the 7½-hour sponge the lightest, though all were satisfactory in this respect. The loaves made from the 4½-, 5½- and 6½-hour sponges were more similar to the bakery sponge loaves than the others.

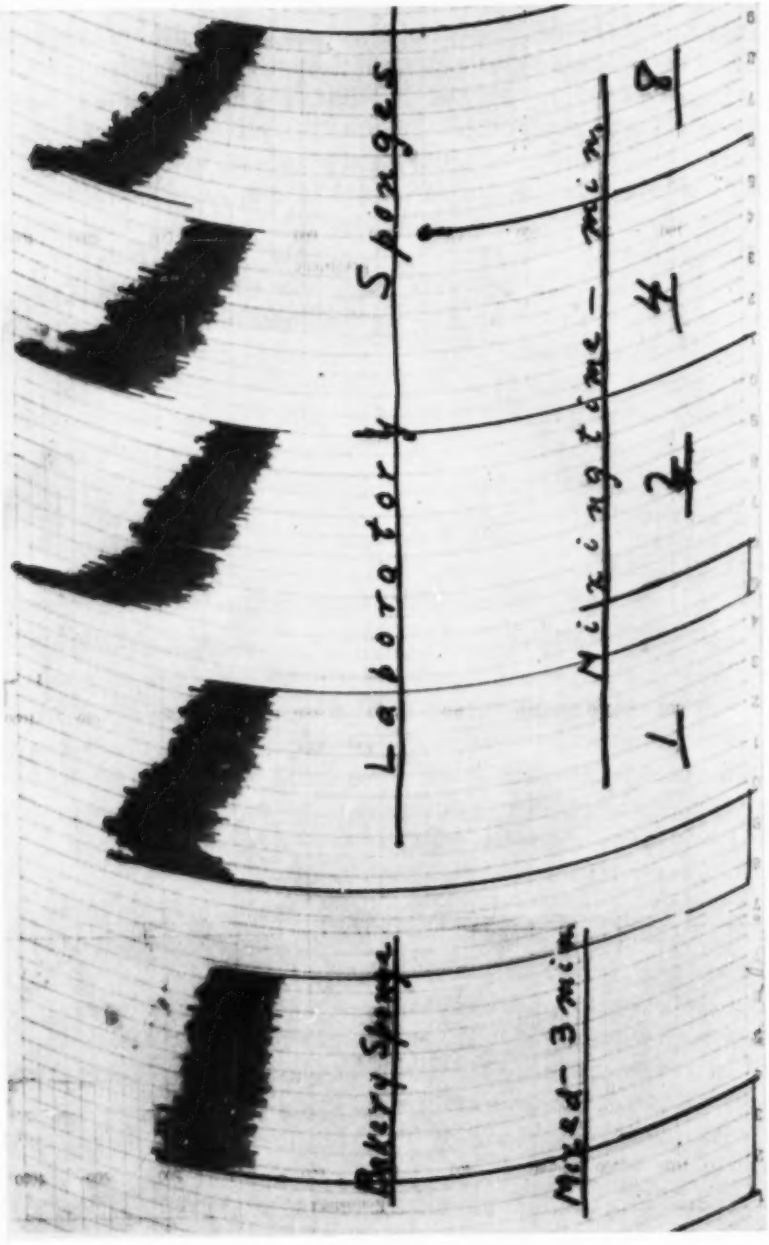


Figure 1. Farinograph curves made from bakery and laboratory sponges mixed for varying lengths of time. These differences, produced in sponges by changes in mixing time, did not affect the resulting bread.

TABLE II

VARIATIONS IN SPONGE FERMENTATION TIME AT 80° F. IN AIR

	Sponge fermentation time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
	Experiment 1			
Large bakery sponge	4½	30	49	2400 [1]
Small laboratory fermented sponge	1½	30	65	2360
	2½	30	61	2370
	3½	30	55	2400
	4½	30	47	2320
	5½	30	44	2400
	6½	30	44	2350
	7½	30	47	2310
	Experiment 2			
Large bakery sponge	4½	30	49	2470 [1]
Small laboratory fermented sponge	4	30	50	2380
	4½	30	48	2430
	5	30	45	2420
	5½	30	48	2400
	6	30	45	2370
	6½	30	42	2500
	7	30	43	2310

[1] Average of 3 loaves.

In exterior appearance there was a progressive change from young to older appearing bread for the 1½- to 4½-hour sponge loaves, the 1½-hour sponge producing a loaf with smoother, more shiny sides, sharper corners, and more sharply defined and deeper pits on the sides and bottom. The 5½-, 6½- and 7½-hour sponge loaves were about like the bakery sponge loaves—all having a well matured exterior appearance. The 4½-hour sponge loaf appeared to be on the border-line between the young and well matured loaves.

In crumb color, the 1½-, 2½- and 7½-hour sponge loaves were slightly darker than the others, which were about like the bakery sponge loaves.

In texture and grain, the 1½- and 2½-hour sponge loaves were slightly young, with cells rounded, although they were not unsatisfactory. The 3½- and 4½-hour sponge loaves had good texture and close, well-developed grain. The 5½-hour loaf was more like the bakery sponge loaves than any of the others, having good texture and grain. The 6½-hour loaf was similar to, but slightly more open than the bakery sponge loaves, and the 7½-hour loaf, while not unsatisfactory, was still more open-grained.

The indications from experiment 1, Table II, were, therefore, that 4½-, 5½- and 6½-hour laboratory sponge fermentations at 80° F. in air would produce bread similar or equal to that resulting from a 4½-hour fermentation of the large bakery sponge under the same conditions.

In experiment 2, Table II, in which the range of sponge fermentation was smaller, the differences between the loaves were of little significance. The crumb colors, crust colors, and exterior characteristics were more or less alike. In texture and grain, the laboratory sponges fermented from 4½ to 6½ hours, inclusive, produced loaves which were all within the range of good bread, and similar to the bakery sponge loaves. These results, therefore confirmed the findings of experiment 1, Table II, that there is a range of about two hours (from 4½ to 6½ hours) within which laboratory sponges fermented at 80° F. in air would produce bread very similar to that produced from a large bakery sponge fermented for 4½ hours at 80° F.

The figures in Table III were obtained in two experiments in which the laboratory size sponges were fermented in jars, the lower portions of which were set in a water bath maintained at 86° F.

TABLE III

VARIATIONS IN SPONGE FERMENTATION TIME FOR SPONGES SET IN WATER BATH AT 86° F.

	Sponge fermentation time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
	Experiment 1			
Large bakery sponge (in air at 80° F.)	4½	30	50	2420
Small laboratory fermented sponge	1½	30	61	2280
	2½	30	54	2350
	3½	30	50	2400
	4½	30	49	2380
	5½	30	47	2450
	6½	30	49	2380
	7½	30	51	2380
	Experiment 2			
Large bakery sponge (in air at 80° F.)	4½	30	48	2430 [1]
Small laboratory fermented sponge	3½	30	48	2440
	4	30	46	2350
	4½	30	46	2440
	5	30	44	2410
	5½	30	46	2470
	6	30	46	2460
	6½	30	46	2440
	7	30	45	2320

[1] Average of 3 loaves.

The temperature of these small sponges in the water bath increased from 76° to 88° F. after 4½ hours of fermentation.

The results of the two experiments listed in Table III were similar with respect to loaf characteristics. The short-time sponges produced loaves with darker crust color than the older sponges. In crumb color the short sponge loaves were also slightly darker than the others. In exterior appearance, the 1½- to 3½-hour sponge loaves showed indications of young doughs, while the others were similar to the bakery sponge loaves and hardly distinguishable from each other. In texture and grain the 1½- and 2½-hour sponge loaves, though good, had rounded cells and appeared young. The 3½-hour sponge loaves had good, well developed grain, and were similar to the bakery sponge loaves, but perhaps still slightly young. The other loaves were all similar to the bakery sponge loaves, having good, well matured texture and grain.

It may therefore be concluded that after a minimum sponge fermentation of about 3½ to 4½ hours, depending on the temperature of the medium within which they are set, and using the type of flour and formula given above, laboratory sponges may be fermented within a wide range of time—up to 7 hours, and possibly more, and then produce bread of good quality, which will be very similar in character to bread made from large bakery sponges fermented for 4½ hours at 80° F.

Dough Mixing

In the dough mixing experiments, the sponges used were mixed and fermented in the same way, variations in time being made when mixing the sponges with the remaining dough ingredients. Preliminary Farinograph curves indicated that small changes in mixing time produced significant variations in the resulting doughs. (Figures 2 and 3.)

Figure 2 shows the changes in the slopes of the curves made from laboratory doughs mixed 1, 2, 3, 4, 6 and 8 minutes in the Fleischmann mixer and then placed in the Farinograph. The differences in feel and consistency of these doughs were quite marked—they were undermixed after 1 and 2 minutes, and too sticky and stringy after 6 and 8 minutes.

Figure 3 shows the similarities between the large bakery dough mixed 10 minutes in the bakery mixer, a one-loaf dough mixed 4 minutes in the Fleischmann mixer, and a four-loaf dough mixed 6 minutes in the Fleischmann mixer.

In the experiments recorded in Table IV, dough mixed in the large bakery mixer was taken to the laboratory, divided into laboratory size portions and handled exactly like the laboratory doughs for which mixing times were varied in the laboratory (Fleischmann) mixer.

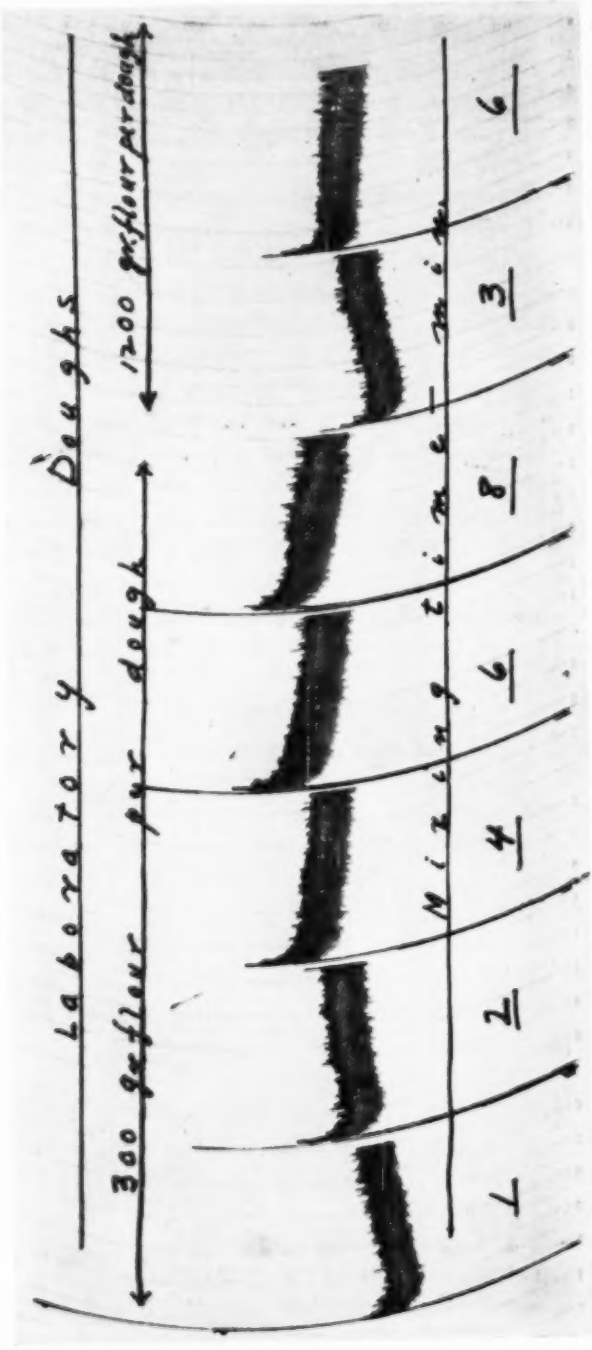


Figure 2. Farinograph curves made from laboratory sponge doughs mixed for varying lengths of time, showing significant differences produced by varying mixing time and size of dough.

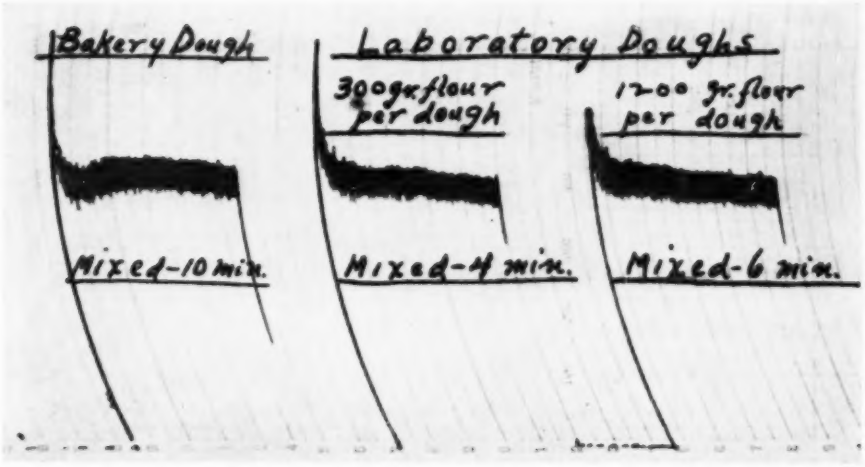


Figure 3. Comparison of Farinograph curves produced by bakery and laboratory sponge doughs, showing mixing time and size of dough in laboratory mixer which gave same mixing result as obtained with large sponge dough in bakery mixer.

The differences between the loaves in experiment 1, Table IV, were very marked, indicating the importance of dough mixing, in which the time range is very small as compared with sponge mixing (Figure 4). The loaf made from the dough mixed 1 minute was poor in volume, crumb color, and texture, having small round holes and thick cell walls. The dough mixed 2 minutes made a loaf which was

TABLE IV
VARIATIONS IN DOUGH MIXING TIME

	Dough mixing time	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Minutes	*Hours*	*Minutes*	*Minutes*	*C.c.*
		Experiment 1			
Bakery dough	10	4½	30	48	2380 [1]
Laboratory dough	1	4½	30	53	1920
	2	4½	30	52	2000
	3	4½	30	49	2280
	4	4½	30	49	2420
	6	4½	30	47	2540
	8	4½	30	46	2520
		Experiment 2			
Bakery dough	10	4½	30	51	2390 [1]
Laboratory dough	3	4½	30	53	2300
	4	4½	30	50	2370
	5	4½	30	52	2380
	6	4½	30	51	2370

[1] Average of 3 loaves.

much better, but still unsatisfactory. The loaf made from the 3-minute mixed dough was nearly equal to the bakery dough loaves in texture, but slightly smaller in volume and darker in crumb color; this loaf, although satisfactory, was still unequal to the bakery dough loaves. The loaf made from the 4-minute mixed dough was equal to the bakery dough loaves in volume, texture and crumb color, indicating that a minimum of 4 minutes of mixing in the laboratory mixer is required to equal 10 minutes of mixing in the bakery mixer. The 6- and 8-minute mixed doughs made loaves which were whiter in crumb color, and softer in texture, indicating that 6- and 8-minute mixing times were too long.

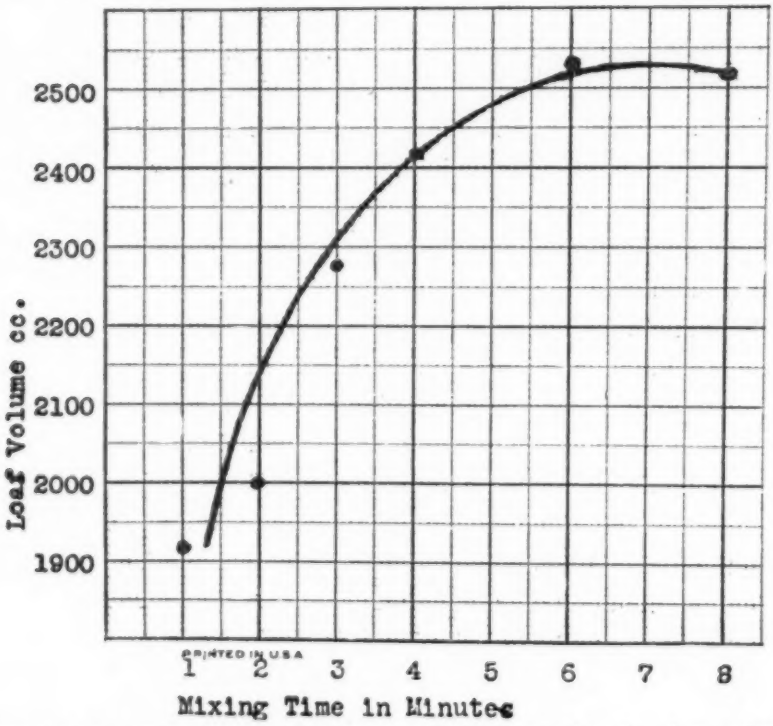


Figure 4. Curve showing changes in loaf volume produced by varying mixing time of sponge doughs in the laboratory mixer.

The loaf characteristics in experiment 2, Table IV, also indicated that 4 minutes of mixing in the laboratory mixer approximated the results of a 10-minute mixing in the bakery mixer more closely than any other mixing time.

Thus, the results of these experiments confirmed the indications of the Farinograph, all pointing to the importance of dough mixing as one of the most critical steps in sponge dough process of breadmaking.

Dough Fermentation

In the dough fermentation experiments, bakery mixed dough was taken to the laboratory and divided into small laboratory size portions, which were fermented for varying periods of time and compared to the large bakery dough which was fermented for 15 minutes, after which portions of it were taken to the laboratory and handled the same way as the others.

The results of two dough fermentation experiments are given in Table V.

TABLE V

VARIATIONS IN DOUGH FERMENTATION TIME

	Dough fermentation time	Sponge time	Pan proof	Volume per pound of bread
	Minutes	*Hours*	*Minutes*	*C.c.*
Experiment 1—Doughs fermented at room temperature				
Large dough, fermented in bakery	15	4½	55	2400 [1]
Small dough, fermented in laboratory	5	4½	53	2390
	15	4½	52	2390
	25	4½	53	2410
	30	4½	51	2390
	40	4½	51	2460
	50	4½	52	2420
Experiment 2—Dough fermented at 86° F. in water bath				
Large dough, fermented in bakery	15	4½	57	2420 [1]
Small dough, fermented in laboratory	10	4½	52	2380
	15	4½	55	2360
	20	4½	53	2440
	30	4½	55	2430

[1] Average of 3 loaves.

The loaves were all quite similar in external appearance. The textures were all good, with only slight variations in the direction of finer texture and grain for the doughs with the longer fermentations. The bakery dough fermented for 15 minutes produced loaves which were more similar to those resulting from the shorter laboratory dough fermentations.

Dividing

The figures in Table VI were obtained in a dough-dividing experiment, in which some of the bakery dough was divided by hand, and the resulting loaves compared to loaves made from dough that had gone through the dividing machine.

TABLE VI

VARIATIONS IN DOUGH-DIVIDING METHODS

	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
Dough divided by hand	4½	15	64	2420 [1]
Dough divided by machine	4½	15	64	2420 [2]

[1] Average of 3 loaves.
[2] Average of 5 loaves.

The loaves made from the doughs divided by hand were practically the same as the machine-divided loaves, indicating that the method of dividing the dough has little influence on the character of the resulting bread.

Bench Proof

An experiment in which bench proof periods of 10, 15, 20 and 25 minutes were used indicated that 15 and 20 minutes gave slightly better results than 10 minutes—which period was not quite enough, producing a younger looking loaf—or than 25 minutes, which period was perhaps a little too long, the resulting loaf having a more open grain.

Molding

In a molding experiment in which bakery dough was used, hand molding was compared with machine molding using two different molding machines, the width between rollers being made the same in both machines. After molding, the doughs were all proofed and baked under the same conditions in the bakery. The figures for this experiment are given in Table VII.

TABLE VII

MOLDING METHODS

Method of molding	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
Bakery molding machine	4½	15	72	2440 [1]
Laboratory molding machine	4½	15	74	2430 [2]
Hand molding	4½	15	73	2520 [2]

[1] Average of 6 loaves.
[2] Average of 3 loaves.

The loaves molded in the two machines were quite similar, and decidedly different in character from the hand-molded loaves, indicating the inadequacy of hand molding for sponge doughs.

Oven Tests

Three ovens were used in the baking experiments—the large, electrically heated bakery oven, and two small laboratory ovens. The latter were of identical construction, except that a rotating tray arrangement had been installed in one for more uniform heating conditions. Steam lines were installed in both laboratory ovens, and steam under about one-pound pressure was admitted into the ovens at the beginning of baking. There was no pressure within the oven because of the ventilating holes and fans, but there was sufficient steam to produce the desired effects. Experiments had indicated that the steam could be used during either a part of or the entire baking period, and both methods were used—the former when baking a number of loaves at one time and the latter when continuously placing single loaves into the oven at short intervals.

Two different sized pans and dough weights were used—the laboratory pans with 17 ounces of dough per pan, and the slightly longer bakery pans with 18 ounces of dough per pan. The laboratory pans were used in the rotating tray oven, and the bakery pans in the stationary oven. During the course of the work it became apparent that better results could be obtained by using the longer baking pans in the rotating tray oven; longer trays were therefore installed in that oven, with a consequent improvement in results. This will be discussed later on in greater detail under the heading "Long Pan Loaves Baked in Rotating Tray Oven."

The figures in Table VIII were obtained in two experiments in which bakery doughs in the long pans, baked in the stationary laboratory oven, were taken to the laboratory after proofing or molding, as indicated, and the resulting loaves compared with those baked in the bakery.

TABLE VIII

BAKERY DOUGHS IN LONG PANS, IN STATIONARY LABORATORY OVEN

	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
Experiment 1—Bakery doughs taken to laboratory after pan proof				
Baked in bakery oven	4½	15	72	2440 [1]
Baked in laboratory oven	4½	15	72	2490 [1]
Experiment 2—Bakery doughs taken to laboratory after molding				
Baked in bakery oven	4½	15	71	2460 [1]
Baked in laboratory oven	4½	15	65	2460 [1]

[1] Average of 6 loaves.

In experiment 1, Table VIII, the loaves baked in the laboratory oven were much lighter on the bottom and very much less uniform on top than those baked in the bakery oven. In texture, the bakery loaves were generally better than the laboratory baked loaves, which were distinctly more open grained. Both bakery and laboratory baked loaves showed a sufficient steam effect—a glossy top crust, characteristic of bread baked with steam in the oven.

In experiment 2, Table VIII, the results were similar in exterior appearance, the laboratory baked loaves being lighter on the bottom than the bakery loaves and darker and much less uniform on top, with crust color variations from light to dark depending on the positions of the loaves in the cooler or hotter end of the oven. Both bakery and laboratory baked loaves showed a satisfactory steam effect. In texture the laboratory baked loaves were better than in experiment 1, and more similar to the bakery baked loaves, though not quite the same in character.

In the experiment recorded in Table IX, 17-ounce doughs in the shorter pans were baked in the rotating tray oven and in the bakery oven, using bakery doughs after molding in the bakery.

TABLE IX

BAKERY DOUGHS IN SHORT PANS, IN THE ROTATING TRAY LABORATORY OVEN

	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
Baked in bakery oven after pan proof in bakery	4½	15	66	2400 [1]
Baked in laboratory oven after pan proof in laboratory	4½	15	62	2420 [1]

[1] Average of 3 loaves.

The loaves baked in the rotating tray oven were lighter on the bottom and darker on top than the bakery loaves but were quite uniform in crust color. Both bakery and laboratory baked loaves had good texture and grain but differed in character to some extent.

As a result of these experiments with different type ovens, significant differences were obtained between doughs which, up to the time of baking, had been handled in identically the same way, thus indicating the importance of proper and uniform baking conditions. Poorer results were obtained in the stationary oven, the heating conditions of which were not uniform and which were also different in type as compared with the bakery oven. The rotating tray oven, with a more uniform heating effect, gave better results in crust color and

texture but did not eliminate type differences, particularly in crust color, since it, too, differed from the bakery oven in the type of heat produced.

Further effects of baking in the different ovens are mentioned in succeeding paragraphs, when considering combinations of the different steps in the sponge dough procedure.

Combinations of Different Steps in the Sponge Dough Procedure

Up to this point in these correlation studies, each step in the procedure had been considered individually. The information thus obtained was then used in studying combinations of the various steps.

Sponge Mixing and Fermentation; Dough Mixing and Fermentation

Experiments were made in which a batch of laboratory mixed and fermented sponge dough, large enough to go through the bakery machine divider, was taken to the bakery after dough fermentation in the laboratory and then divided, rounded, molded, proofed and baked in the bakery at the time the large bakery sponge dough was going through these same steps.

In the experiments, data from which are given in Table X, two 4-loaf laboratory doughs were combined after dough mixing, and fermented in the laboratory, then taken to the divider, etc. In one experiment the four-loaf batches were mixed 5 minutes, and in another 7 minutes, in the laboratory mixer; the laboratory sponges used in making these doughs were set in the 86° F. water bath.

TABLE X

LABORATORY SPONGE DOUGHS HANDLED IN BAKERY AFTER DOUGH FERMENTATION

	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
Experiment 1—Laboratory dough mixed 5 minutes				
Complete bakery procedure	4½	15	63	2470 [1]
Sponge and dough mixed and fermented in laboratory	4½	15	63	2300 [1]
Experiment 2—Laboratory dough mixed 7 minutes				
Complete bakery procedure	4½	15	64	2440 [1]
Sponge and dough mixed and fermented in laboratory	4½	15	64	2360 [1]

[1] Average of 5 loaves.

In experiment 1, Table X, the loaves made from the laboratory dough were smaller in volume and darker in crumb color than the bakery loaves, but were otherwise quite similar to the bakery bread in texture, grain, and exterior characteristics.

In experiment 2, Table X, in which the laboratory dough was mixed longer, the loaves were better in volume than in experiment 1, and slightly whiter in crumb color than the bakery loaves; in texture and grain the laboratory dough loaves were similar to and hard to distinguish from the bakery loaves. It is therefore apparent that the smaller volume and darker crumb color of the laboratory dough loaves in the first experiment were due to insufficient dough mixing, and that the whiter crumb color of the laboratory dough loaves in the second experiment was due to slightly too much dough mixing.

Farinograph curves (Figure 3) had indicated that 6 minutes of mixing would produce the equivalent of bakery mixed dough when mixing a four-loaf batch in the laboratory. The above experiments, in which 5 minutes of mixing were not enough and 7 minutes a little too much for a four-loaf laboratory dough batch, confirmed the indications of the Farinograph.

In the experiments recorded in Table XI, the four-loaf laboratory dough batches were therefore mixed 6 minutes. One of these doughs was made from a laboratory sponge fermented in air at 80° F., and another dough was made from a laboratory sponge fermented in the water bath at 86° F. After dough mixing and fermentation in the laboratory, these doughs were taken to the bakery and put through bakery machinery in the usual way, then proofed and baked in the bakery. The resulting loaves were compared with those made from a portion cut from large bakery dough—equal in weight to the laboratory doughs and handled in exactly the same way.

TABLE XI

LABORATORY SPONGE DOUGHS, HANDLED IN BAKERY AFTER DOUGH FERMENTATION

		Sponge time	Dough time	Pan proof	Volume per pound of bread
		Hours	*Minutes*	*Minutes*	*C.c.*
Complete bakery procedure (small portion from large bakery dough)		4½	20	67	2410 [1]
Sponge and dough mixed and fermented in laboratory; dough mixed 6 minutes	Sponge set in air at 80° F.	5¼	20	67	2410
	Sponge set in water bath at 86° F.	4½	20	67	2360 [1]

[1] Average of 4 loaves.

The loaves made from the laboratory sponge doughs were very much like the bakery loaves in exterior appearance and crumb color. In texture and grain the laboratory dough loaves were of the same general character as the bakery loaves, but slightly closer grained and more uniform. (Figures 5 and 6.)

These experiments, therefore, showed that if laboratory sponge doughs, after proper mixing and fermentation, were taken to the bakery and then handled according to the usual bakery procedure, the resulting laboratory dough bread would be practically equivalent to bread made from sponge doughs that were mixed, fermented, and handled entirely in the bakery.

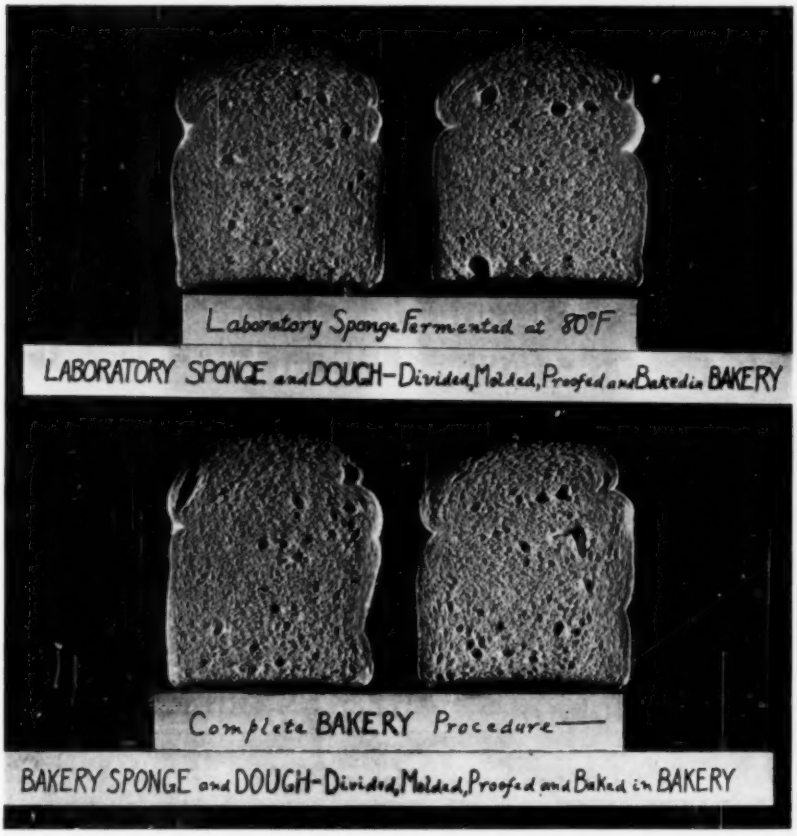

Laboratory Sponge Fermented at 80°F
LABORATORY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY
Complete BAKERY Procedure
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 5. Bread made from laboratory sponge dough, handled and baked in bakery after laboratory mixing and fermentation, compared to bakery bread; laboratory sponge fermented at 80° F. in air.

Laboratory Sponge and Dough Handled Entirely in the Laboratory

Experiments were then made in which the sponge and dough were mixed, fermented, and handled completely in the laboratory. At the same time, some of the laboratory sponge doughs were taken to bakery after molding to be baked and compared with dough handled entirely in the bakery. Similarly, bakery sponge doughs were taken to the laboratory after mixing or molding to be handled, baked and compared

with dough handled entirely in the laboratory. Short and long pans were used in both the bakery and the laboratory, the short pan doughs being baked in the rotating tray oven, and the long pan doughs in the stationary oven.

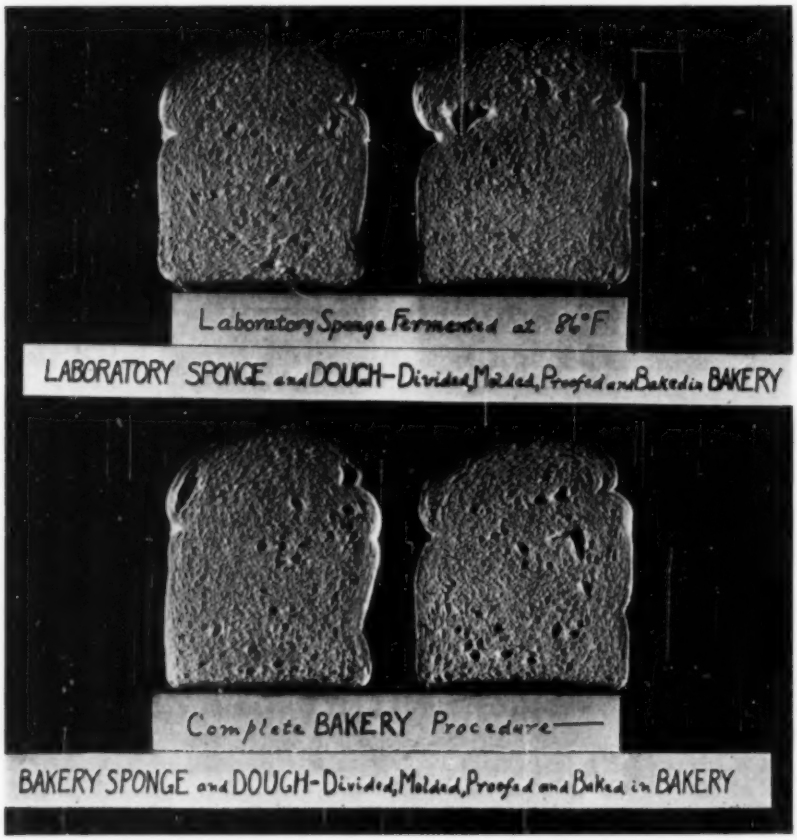
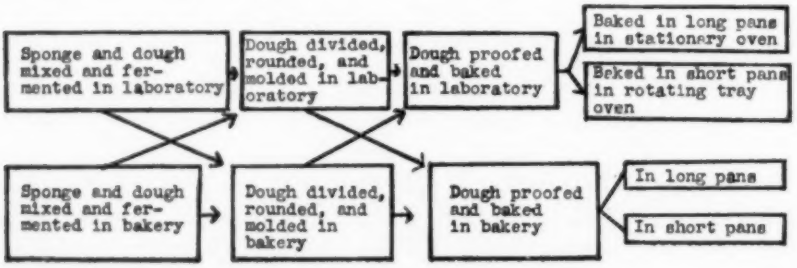

Laboratory Sponge Fermented at 86°F
LABORATORY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY
Complete BAKERY Procedure
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 6. Bread made from laboratory sponge dough, handled and baked in bakery after laboratory mixing and fermentation, compared to bakery bread; laboratory sponge fermented at 86° F. in water bath.

The following diagram indicates what was done:


Sponge and dough mixed and fermented in laboratory
Dough divided, rounded, and molded in laboratory
Dough proofed and baked in laboratory
Baked in long pans in stationary oven
Baked in short pans in rotating tray oven
Sponge and dough mixed and fermented in bakery
Dough divided, rounded, and molded in bakery
Dough proofed and baked in bakery
In long pans
In short pans

The data for one of these experiments are given in Table XII.

TABLE XII

COMPARISON OF COMPLETE AND PARTIAL BAKERY AND LABORATORY PROCEDURES

Sponge time	Dough time	Pan proof	Volume per pound of bread
Hours	*Minutes*	*Minutes*	*C.c.*
	Complete laboratory procedure; baked in long pans in stationary oven		
4½	20	65	2520 [1]
	Complete laboratory procedure; baked in short pans in rotating tray oven		
4½	20	62	2410 [1]
	Laboratory sponge dough, proofed and baked in bakery, in long pans		
4½	20	67	2550 [1]
	Complete bakery procedure; baked in long pans		
4½	20	68	2500 [1]
	Complete bakery procedure; baked in short pans		
4½	20	69	2420 [1]
	Bakery sponge dough, proofed and baked in laboratory; baked in long pans in stationary oven		
4½	20	74	2470 [1]
	Bakery sponge dough, proofed and baked in laboratory; baked in short pans in rotating tray oven		
4½	20	66	2250 [1]

[1] Average of 4 loaves.

The loaves baked in the long pans from dough made and handled completely in the laboratory were similar in internal characteristics to the bakery loaves, but more open grained. The loaves made from the bakery dough, which were baked in the long pans in the laboratory oven, were also more open grained than the bakery loaves, being quite similar to the laboratory loaves in that respect, and indicating that conditions in the stationary oven, in which both were baked, tended to produce a more open grain than the bakery oven.

On the other hand, the short pan loaves baked in the rotating tray oven from dough made and handled completely in the laboratory were closer grained and had slightly finer texture than the short pan bakery loaves. There was also a greater similarity between the bakery dough loaves baked in the rotating tray and bakery ovens, than between those baked in the stationary and bakery ovens. These results indicated that either the shorter pan or more uniform baking conditions, or both, tended to give better results in the rotating tray oven.

In crust color, the loaves baked in the rotating tray oven were more uniform than those baked in the stationary oven, but both produced lighter bottom crusts than the bakery oven.

The figures for another similar experiment are given in Table XIII.

TABLE XIII

COMPARISON OF COMPLETE AND PARTIAL BAKERY AND LABORATORY PROCEDURES

Sponge time	Dough time	Pan proof	Volume per pound of bread
Hours	*Minutes*	*Minutes*	*C.c.*
	Complete laboratory procedure; baked in long pans in stationary oven		
4½	20	67	2520 [1]
	Complete laboratory procedure; baked in short pans in stationary oven		
4½	20	59	2480 [1]
	Laboratory sponge and dough, proofed and baked in bakery, in long pans		
4½	20	69	2570 [2]
	Laboratory sponge and dough, proofed and baked in bakery, in short pans		
4½	20	69	2500 [2]
	Complete bakery procedure; baked in long pans		
4½	20	68	2540 [1]
	Complete bakery procedure; baked in short pans		
4½	20	68	2440 [1]
	Bakery sponge and dough, divided, rounded, molded, proofed and baked in laboratory; baked in long pans in stationary oven		
4½	20	69	2500 [1]
	Bakery sponge and dough, divided, rounded, molded, proofed and baked in laboratory; baked in short pans in rotating tray oven		
4½	20	59	2430 [1]

[1] Average of 4 loaves.
[2] Average of 2 loaves.

In internal characteristics, the long pan loaves made completely in the laboratory were generally more open grained and not as good as the long pan bakery loaves. The long pan loaves made from the bakery doughs which were handled and baked in the laboratory were very much like the laboratory loaves, and quite different from the bakery loaves, although the latter were made from identically the same dough. Similarly, the long pan loaves made from laboratory dough and baked in the bakery resembled the bakery loaves much more than the laboratory loaves.

The short pan loaves showed greater similarities than the long pan loaves. Those made from the laboratory doughs and baked in the stationary oven had surprisingly good texture and grain, in contrast to

long pan loaves baked in the same oven, indicating that the shape of the pan may also influence the character of the resulting bread. The bakery short pan loaves also had good texture and grain, and were similar to the laboratory loaves, but not quite the same. The bakery doughs handled and baked in short pans in the laboratory were distinctly more similar to the laboratory than to the bakery short pan loaves. The most striking similarities were obtained between the short pan bakery loaves and those made from laboratory dough which were proofed and baked in similar pans in the bakery; they were identically the same in texture and grain, and could not be distinguished from each other in the slightest degree.

These results again emphasized the marked influence of oven conditions, which produced differences between doughs which were otherwise identically the same, when baked in different ovens, and produced identically the same type of bread from doughs which had been handled differently up to the time of baking.

In the above experiments, the laboratory dough batches had been made from 1200 g. of flour—enough to make 4 loaves. In the experiment shown in Table XIV, single-loaf batches were made in the laboratory.

TABLE XIV

COMPARISON OF COMPLETE AND PARTIAL BAKERY AND LABORATORY PROCEDURES

Sponge time	Dough time	Pan proof	Volume per pound of bread
Hours	*Minutes*	*Minutes*	*C.c.*
	Complete laboratory procedure; baked in long pans in stationary oven		
4½	20	64	2510
4½	20	64	2540
4½	20	65	2500
4½	20	68	2500
	Laboratory sponge and dough, proofed and baked in bakery, in long pans		
4½	20	68	2570
	Laboratory sponge and dough, proofed and baked in bakery, in short pans		
4½	20	67	2540
	Complete bakery procedure; baked in long pans		
4½	20	68	2460 [1]
	Complete bakery procedure, baked in short pans		
4½	20	68	2420 [1]
	Bakery sponge and dough, proofed and baked in laboratory; baked in long pans in stationary oven		
4½	20	69	2510 [1]

[1] Average of 4 loaves.

The results of this experiment with single-loaf laboratory doughs were similar to those in which the four-loaf laboratory doughs had been used. The long pan laboratory loaves were much more open grained than the bakery loaves, and very much like the long pan loaves that had been made from bakery dough and baked in the laboratory oven. The laboratory dough loaf baked in the bakery was more similar to the

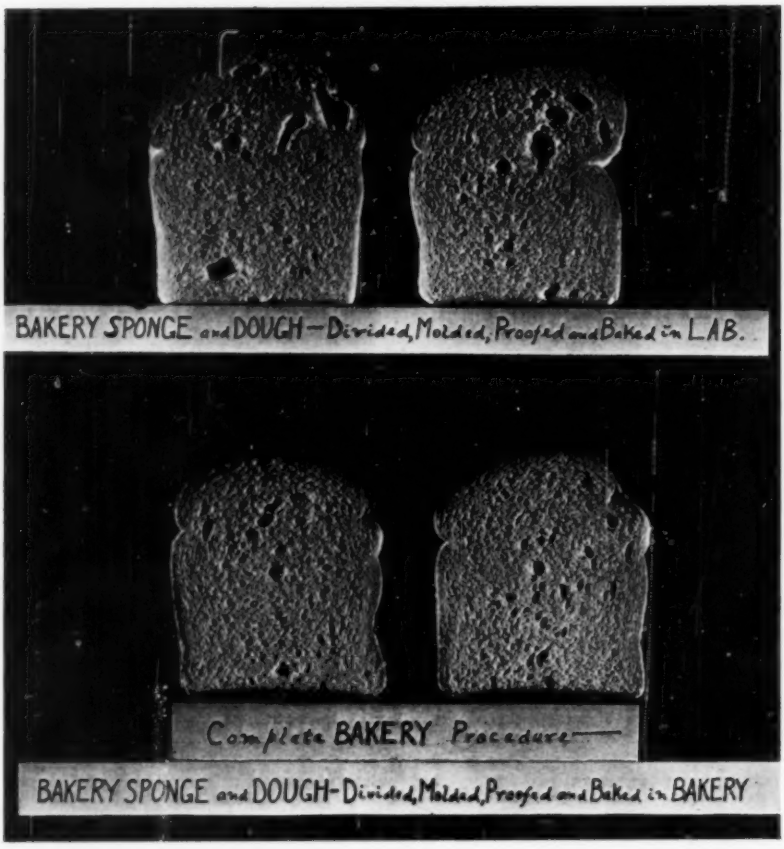

BAKERY SPONGE and DOUGH—Divided, Molded, Proofed and Baked in LAB.
Complete BAKERY Procedure—
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 7. Bakery sponge dough, handled and baked in laboratory after mixing and fermentation, has open grain and large holes, compared with bread made entirely in bakery.

bakery than to the laboratory loaves. The short pan loaf made from laboratory dough baked in the bakery was, again, identically the same in texture and grain as the short pan bakery loaves. (Figures 7, 8, 9, 10, 11, 12, and 13).

It is apparent from the photographs (Figure 8) that the loaves baked in the stationary laboratory oven had some unusually large

holes. This may be taken to indicate the possibility that oven conditions are a contributing factor to "holes in bread," a possibility which has perhaps been overlooked in seeking the causes of this troublesome problem.

An interesting observation was made on the crust color of the loaves baked in the stationary laboratory oven in the experiment

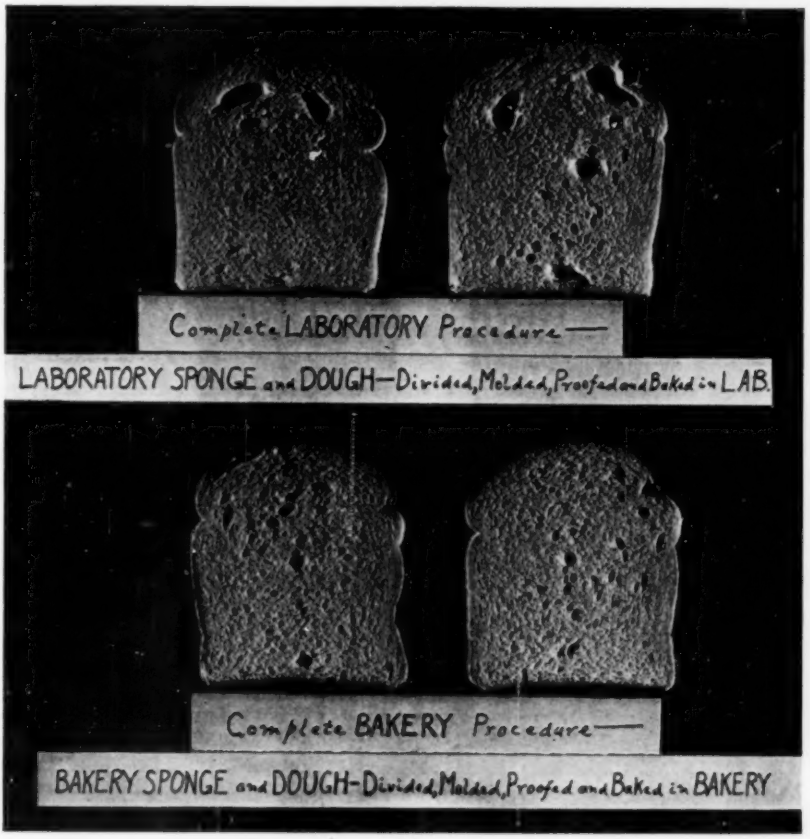

Complete LABORATORY Procedure—
LABORATORY SPONGE and DOUGH—Divided, Molded, Proofed and Baked in LAB.
Complete BAKERY Procedure—
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 8. Bread made entirely in the laboratory has open grain and large holes, compared with bakery bread.

with the single-loaf doughs. The four loaves made from the bakery dough, which were all baked in this oven at the same time, showed the usual wide variations in color of the top crust. But the single loaf laboratory doughs, which went into the oven one at a time within short intervals, were shifted by hand from one end of the oven to the other, so that each loaf was subjected to approximately the same oven

conditions. The resulting crust colors were consequently very much more uniform. (Figure 14.)

Long Pan Loaves Baked in Rotating Tray Oven

The above experiments had indicated that better results were obtained in the rotating tray oven with the shorter pans. In order to see whether similar results could be obtained with the long pans, longer

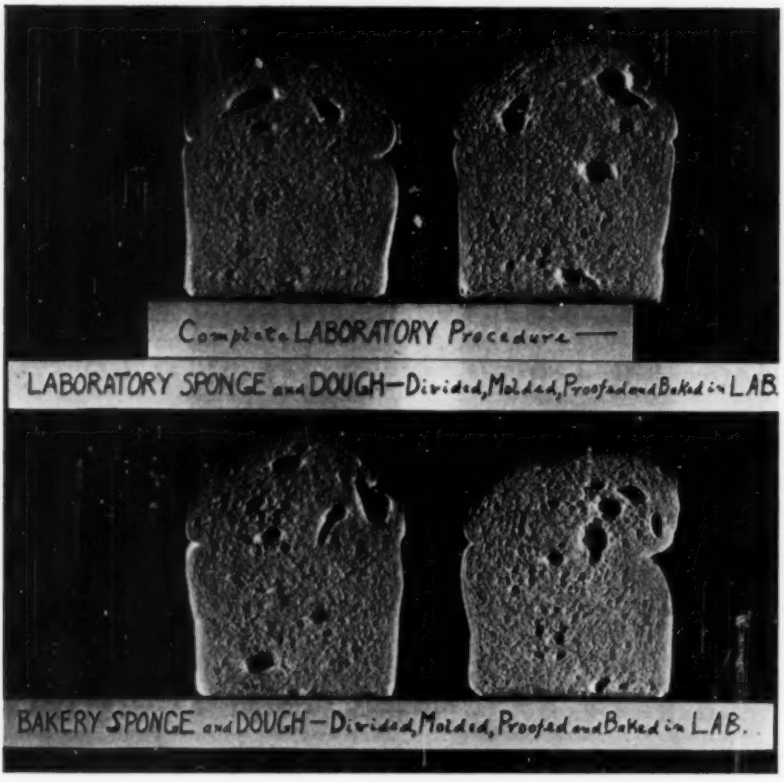

Complete LABORATORY Procedure —
LABORATORY SPONGE and DOUGH—Divided, Molded, Proofed and Baked in LAB.
BAKERY SPONGE and DOUGH—Divided, Molded, Proofed and Baked in LAB.

Figure 9. This figure shows the similarity between the bread made entirely in the laboratory and that made from bakery dough, but handled and baked in the laboratory.

trays were installed in the rotating tray oven, and doughs made and handled completely in the laboratory were then baked in long pans under these improved oven conditions, and compared with bakery doughs baked in both bakery and laboratory ovens.

The figures for two experiments are shown in Table XV.

The change from the stationary to the rotating tray oven when baking the long pan loaves produced a marked improvement in the

texture and grain of the resulting bread. The laboratory loaves were much more similar to the bakery loaves than they had been when using the stationary oven. In experiment 2 the laboratory loaves were quite as good as the bakery loaves in texture and grain, and differences as to type were hardly apparent in that respect. (Figure 15.)

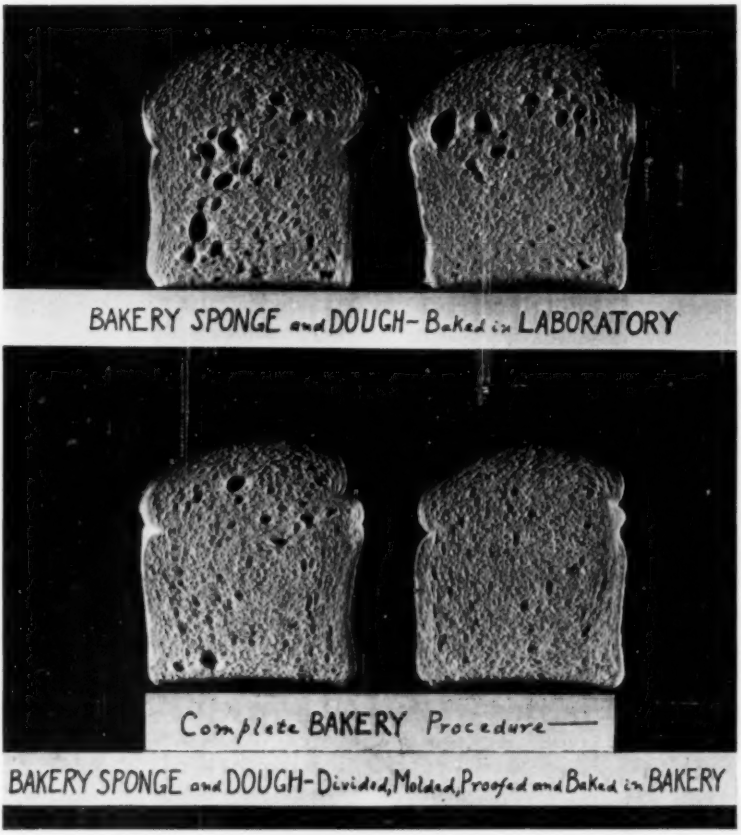

BAKERY SPONGE and DOUGH- Baked in LABORATORY
Complete BAKERY Procedure——
BAKERY SPONGE and DOUGH- Divided, Molded, Proofed and Baked in BAKERY

Figure 10. Bakery sponge dough, baked in laboratory after mixing, fermentation and handling in the bakery, has open grain and large holes compared with bread made entirely in the bakery; laboratory baking was the only variable which produced these differences.

In crust color, also, a distinct improvement was apparent. The laboratory loaves were much more uniform than they had been in the stationary oven. There were, of course, slight differences, such as darker side and bottom crusts on the bakery bread, but these were due to inherent differences between the ovens rather than to faulty baking. Both bakery and laboratory loaves were well baked and entirely satisfactory, the differences between them being no greater than those that

might be obtained between bread baked in different types of commercial ovens.

Summary and Discussion

Using a commercial type formula, and the same ingredients in the bakery and laboratory, a study was made of the different steps involved in the sponge dough procedure. The findings with respect to the different steps were as follows:

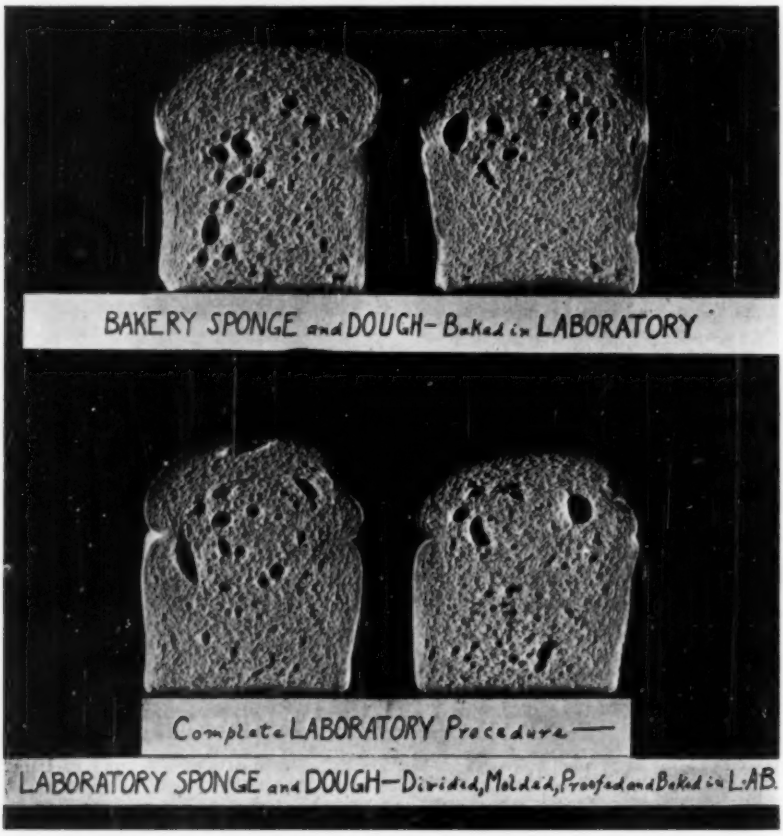

BAKERY SPONGE and DOUGH-Baked in LABORATORY
Complete LABORATORY Procedure—
LABORATORY SPONGE and DOUGH—Divided, Molded, Proofed and Baked in LAB.

Figure 11. Bakery sponge dough, baked in laboratory, compared with bread made entirely in the laboratory, showing similarities in texture and grain.

Sponge mixing.—Sponges were mixed for varying periods of time, without producing any apparent differences in the resulting bread. There seems to be considerable leeway in this respect. In practice, sponges are mixed just long enough to get a homogeneous mixture of ingredients, without necessarily producing any dough development.

Sponge fermentation.—When fermented alongside of each other at the same dough-room temperature, small laboratory sponges required a longer fermentation period than the large bakery sponge, in order to produce the same results. But when fermented in a water bath at a higher temperature than that of the room in which the large sponge was fermented, the small laboratory sponges produced results equal to those of the large bakery sponge in the same fermentation period.

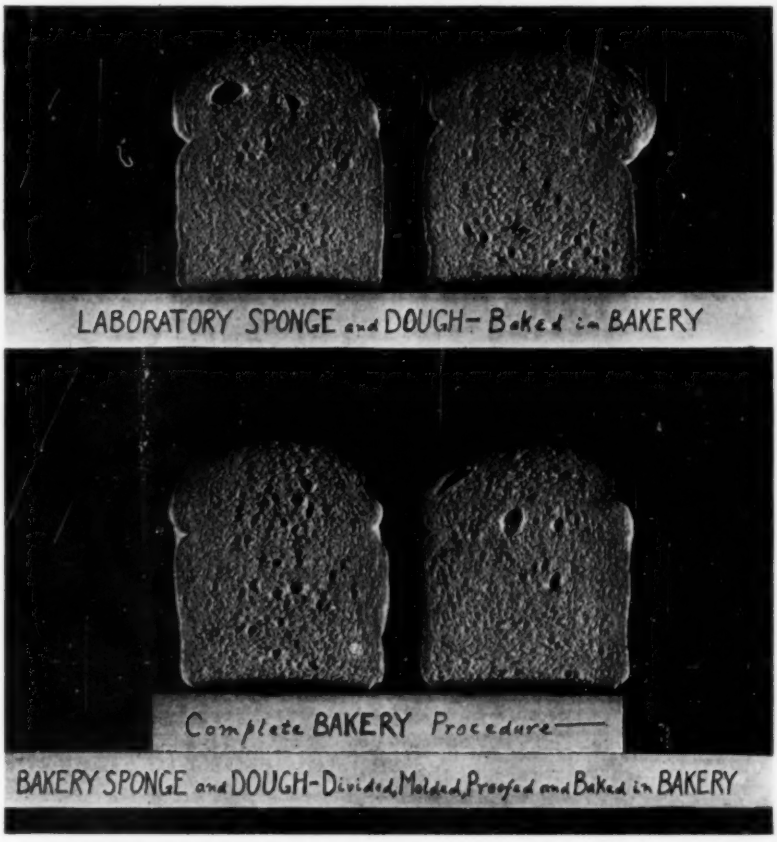

LABORATORY SPONGE and DOUGH-Baked in BAKERY
Complete BAKERY Procedure—
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 12. Laboratory sponge dough, baked in bakery compared with bakery bread, showing elimination of open grain and large holes, by changing from stationary laboratory oven to bakery oven.

Dough mixing.—When mixing the laboratory sponges with the remaining dough ingredients after sponge fermentation, it was necessary to mix 4 minutes for a one-loaf dough and 6 minutes for a four-loaf dough in the laboratory mixer in order to duplicate 10 minutes of mixing for the large sponge dough in the bakery mixer. Dough mixing

proved to be a much more critical step in the procedure than sponge mixing or sponge fermentation. One minute differences in mixing time on either side of the correct mixing interval produced noticeable differences in the resulting bread. The correct mixing interval seems to be close to but slightly below that which produced maximum loaf volume.

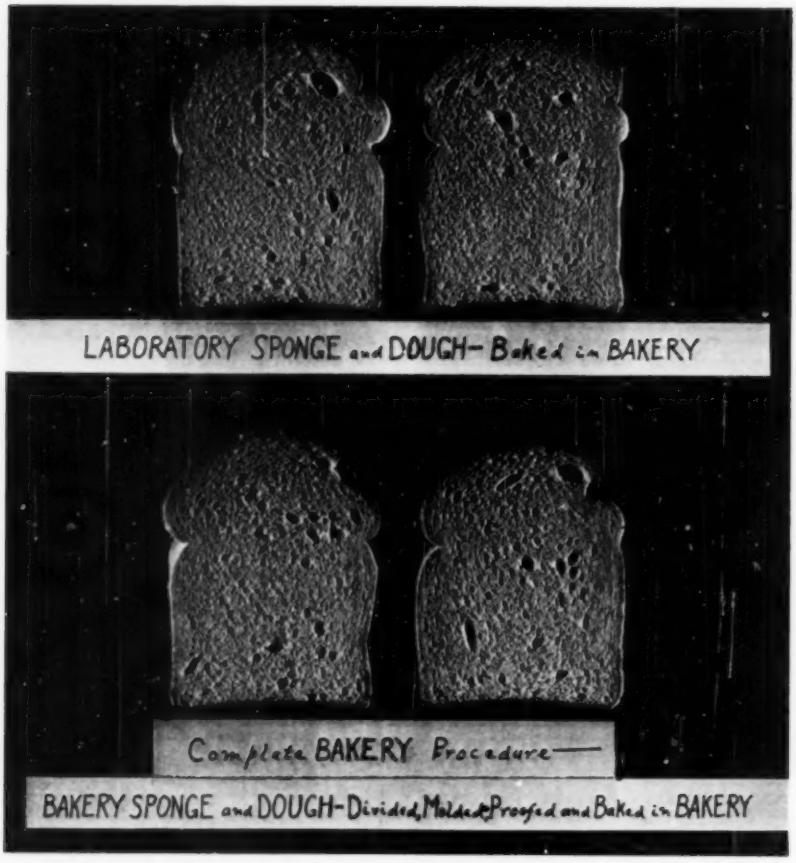

LABORATORY SPONGE and DOUGH-Baked in BAKERY
Complete BAKERY Procedure—
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 13. Comparison same as in Figure 12, except that bread was baked in shorter pans.

Dough fermentation.—A short fermentation period of about 20 to 25 minutes in the laboratory produced results similar to those obtained in the bakery with a 15- to 20-minute fermentation period. Longer fermentation times produced good bread, slightly better in texture and grain than that resulting from the shorter fermentation, but the differences were noticeable only on close inspection, and the doughs

TABLE XV

LONG PANS IN ROTATING TRAY OVEN

	Sponge time	Dough time	Pan proof	Volume per pound of bread
	Hours	*Minutes*	*Minutes*	*C.c.*
Complete laboratory procedure; baked in long pans in rotating tray oven				
Experiment 1				
Four-loaf dough batches	4½	20	67	2500 [1]
Single-loaf dough batches	4½	20	66	2600
	4½	20	66	2640
	4½	20	66	2620
	4½	20	67	2640
Experiment 2				
Four-loaf dough batches	4½	20	65	2480 [1]
Single-loaf dough batches	4½	20	65	2530
	4½	20	67	2540
	4½	20	66	2540
Complete bakery procedure; baked in long pans				
Experiment 1				
	4½	20	74	2540 [1]
Experiment 2				
	4½	20	75	2470 [1]
Bakery sponge and dough, proofed and baked in laboratory, baked in long pans in rotating tray oven				
Experiment 1				
	4½	20	73	2580 [2]
Experiment 2				
	4½	20	72	2440 [1]

[1] Average of 4 loaves.
[2] Average of 3 loaves.

were more lively and not as smooth when going through the molding machine.

Dividing, rounding, bench proof, and pan proof.—These steps between fermentation and baking, when performed in either bakery or laboratory, produced no differences of real significance.

Molding.—Doughs molded in two different machines produced loaves which were very much alike, but entirely different from those made as a result of hand molding.

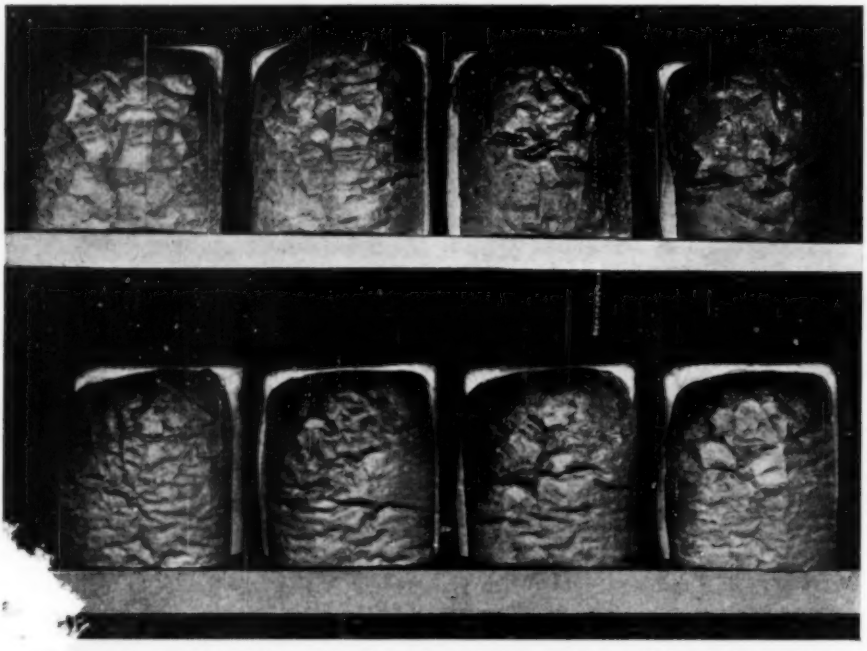


Figure 14. Bread baked in stationary laboratory oven; top row loaves all baked at the same time; bottom row loaves placed in oven one at a time at short intervals, and shifted from one end of the oven to the other.

Baking.—Significant differences were produced in baking the same doughs in different ovens. Marked improvements in the internal and external characteristics of the laboratory bread were produced by baking in a rotating tray oven, which also eliminated type differences in internal appearance to a large extent.

Combinations of the different steps in the sponge dough procedure.—Bread made from sponge doughs which were put through every step in the procedure completely in the laboratory, was entirely satisfactory in every respect, being similar in internal character to bread made in the bakery with the same ingredients and formula, and not much different

externally. Any external differences were due to oven variations and not to faulty baking.

While our results were obtained in a given laboratory and plant under a particular set of conditions and cannot literally be applied to any other case, the findings should prove capable of fairly general application in serving as a guide in other plant-laboratory correlations. After determining the minimum amounts of sponge mixing or fermenta-

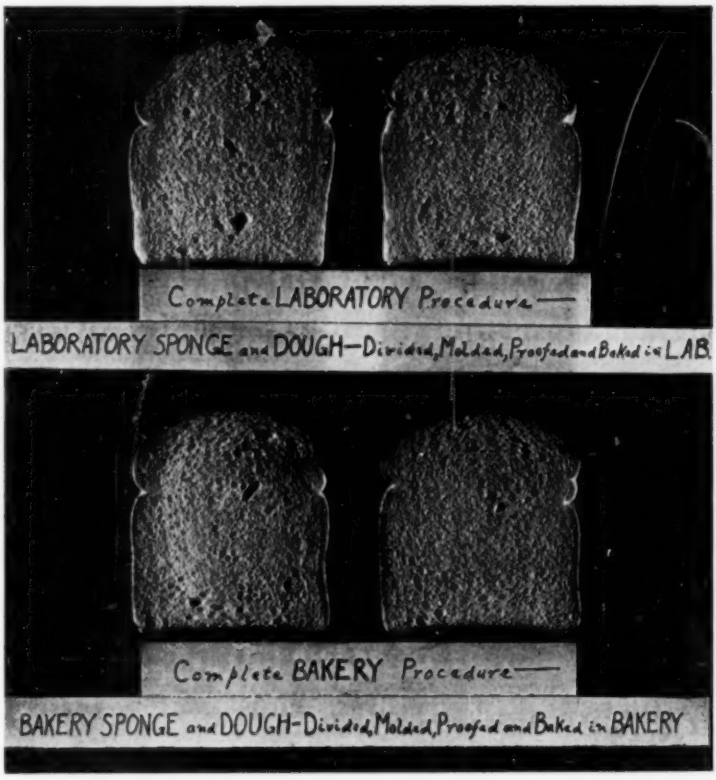

Complete LABORATORY Procedure —
LABORATORY SPONGE and DOUGH—Divided, Molded, Proofed and Baked in LAB.
Complete BAKERY Procedure —
BAKERY SPONGE and DOUGH-Divided, Molded, Proofed and Baked in BAKERY

Figure 15. Bread made entirely in laboratory compared with bakery bread, showing elimination of large holes and open grain by changing from stationary to rotating tray laboratory oven.

tion in a given case, continued mixing or fermentation over a fairly wide range of time should make no significant differences in the resulting bread. Dough mixing was found a critical factor in our work, and requires careful study; good results were obtained when using the mixing time which produced close to maximum loaf volume; a few carefully conducted tests should indicate the proper mixing interval in any other situation. Dough fermentation for a short period of time

gave the desired results; a minimum of about 20 minutes is satisfactory, and longer fermentations also give good results but are not generally used. Dough dividing and rounding provided no particular difficulties when done by hand, and produced results similar to those resulting from machine dividing and rounding; this should be found true in other cases. We found hand molding entirely unsatisfactory in producing bread similar in appearance to commercial bread; any molding machine which has a sufficient range of adjustments should prove satisfactory. Proofing in the pan should provide no difficulties, other things being satisfactory (Davis, Leatherock, and Putnam, 1936).[4] We found oven conditions to be very important in their effects on the internal and external characteristics of the resulting bread. While exact duplication of baking results between laboratory and commercial ovens may be very difficult, uniform heating conditions, aided by a rotating arrangement in the oven, should give satisfactory results in producing a loaf similar in appearance to a typical commercial loaf.

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[4] Davis, C. F., Leatherock, L. E., and Putnam, H. W. Experimental baking pans. Cereal Chem. **13**: 113–118 (1936).

QUALITY IN DURUM WHEATS AND A METHOD FOR TESTING SMALL SAMPLES

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Introduction

Durum wheat is an important crop in North Dakota, South Dakota, Minnesota, and Montana. Since shortly after its introduction in the United States, about 1900, durum wheat has been grown on from three to five million acres annually in those four States, with an average production between 30,000,000 and 50,000,000 bushels. Approximately half this quantity is required annually for the manufacture of high grade macaroni consumed in this country. The quality requirements of durum wheat used in making macaroni are very exacting. Consequently, any experimental method for evaluating durum wheat quality must reflect accurately the behavior of a variety under actual commercial manufacturing conditions.

It, therefore, has been the aim of research workers to perfect testing methods similar to the commercial procedure because the actual making of macaroni is the final test of quality for any durum sample. In general laboratory practice, at least five pounds of grain and in some cases more are required to make a test. Such samples are available from varietal plot experiments. In certain cases, however, it is desirable to determine the quality of much smaller samples, particularly from durum wheat breeding nurseries where less than a pound of grain is available.

The method described here requires only 100 g. of wheat for milling into semolina which then is processed into a macaroni disc, similar in almost every respect to the regular tubular type macaroni, except for shape. By this test many small nursery samples may be determined for quality in a fraction of the time required by methods now in use.

Variation in Quality of Durum Wheat Samples

Samples of durum wheat vary greatly in their suitability for making macaroni due both to variety and to the conditions under which the grain was grown. Long experience by the macaroni manufacturers has

[1] Division of Cereal Crops and Diseases, Bureau of Plant Industry.
[2] Grain Division, Bureau of Agricultural Economics.
[3] The writers wish to express appreciation to D. A. Coleman and J. Allen Clark for advice and assistance given.

resulted in the practice of offering premiums for high quality amber durum varieties, such as Mindum and Kubanka, while low quality varieties such as Pentad (red durum), Monad, and Acme, grown in the same locality, bring a low price.

A comparison of samples of macaroni from a number of standard durum varieties grown at the Langdon Substation, Langdon, North Dakota, has been made from the seven crops grown during the period from 1929 to 1935. The durum wheats were grown side by side in narrow 1/60-acre plots for the purpose of determining yields and other agronomic comparisons. Each year samples of the varieties were sent to the experimental mill of Washburn-Crosby Company, Minneapolis, where the grain was milled into semolina and processed into macaroni. The samples were identified by letter only, until the final report was submitted. Thus the commercial men were asked to rank the varieties processed each year according to the commercial desirability of the macaroni each produced.

The relative rank of each variety in the different years is tabulated in Table I. The uniformity of the ranking from year to year is very

TABLE I

RELATIVE RANKING OF DURUM WHEAT VARIETIES FOR QUALITY OF MACARONI MADE BY THE WASHBURN-CROSBY COMPANY EXPERIMENTAL LABORATORY FROM DURUM GROWN AT THE LANGDON SUBSTATION FROM 1929 TO 1935 (RANKED WITHOUT KNOWLEDGE OF THE IDENTITY OF THE SAMPLES)

Variety	C. I. number	Year tested and rank for quality of macaroni							
		1929	1930	1931	1932	1933	1934	1935	Rank
Mindum	5296	1	1	1	2	1	1	1	1
Kubanka—75	11541	2	2	2	1	2	2	2	2
Akrona	6881	3	3	3	3	–	–	–	3
Kubanka—132	8383	–	4	5	4	3	3	4	4
Kubanka	1440	–	5	4	5	4	4	3	5
N.D.R. 216	—	–	6	6	6	–	–	–	6
Nodak	6519	–	7	7	–	–	–	–	7
Golden Ball	11477	–	8	8	8	6	5	5	8
Monad	3320	–	9	9	7	5	6	6	9
		25.3[1]	40.5[1]	24.6[1]	32.2[1]	11.8[1]	24.3[1]	8.9[1]	23.9[1]

[1] Average yield per acre (bushels).

striking. Thus, either Mindum or Kubanka—75 made the best macaroni, while either Golden Ball or Monad made the poorest. Furthermore, Kubanka—132 and Kubanka (C. I.[4] 1440) were always intermediate. The significance of these results is emphasized when one considers the wide range of climatic conditions in the different seasons,

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

as reflected in the yields of the varieties grown. This does not mean that environment is not important in the growing of durum with good milling and macaroni quality, for it is well known that some localities sown with the best variety never produce good macaroni wheat, while others almost invariably do. Data in the table indicate that under comparable soil conditions, some varieties have a hereditary capacity for making better macaroni than others, even when conditions vary widely from year to year.

The effect of environment on quality of durum wheat is perhaps more generally appreciated than the effect of variety. To illustrate this effect, an example may be drawn from records in the Department of Agriculture. Each year in the experimental macaroni laboratory, located in Washington, D. C., samples of durum from the varietal plots of some 20 Great Plains experiment stations are processed into macaroni and the color analyzed on a disc colorimeter. In general, the samples with the highest readings of yellow are preferable from the quality standpoint. In 1932 the percentages of yellow in the macaroni from Mindum and Monad grown at Langdon, North Dakota, were 35.5 and 24.5 respectively. At several of the stations outside the durum wheat area, the macaroni from Mindum (the superior variety) gave readings of less than 25% yellow, or poorer than the undesirable Monad grown in a favorable durum area.

Accepted Methods of Measuring Durum Wheat Quality

In attempting to evaluate the quality of small samples of durum wheat, a number of tests have been employed. In general, these tests have been useful in describing certain characteristics of the sample but have failed either individually or collectively to give a very complete picture of its probable performance in the macaroni test.

One of the oldest methods of estimating durum quality is simply a visual inspection of the seed. Wide differences in the appearance of the kernel do exist and often appear to be correlated with the quality of the resulting macaroni, but differences of opinion, even between experienced durum buyers, indicate that this method needs to be checked constantly by making macaroni. Furthermore, it is possible that an undesirable color of the bran coat may sometimes mask a desirable endosperm, even in amber varieties.

Another method is by means of wet and dry slicks of semolina. Here again the method is useful only in the hands of an experienced operator, and the best looking semolina does not always produce the best macaroni.

The protein test is generally accepted as a useful index of quality and should be a routine determination in any study of durum wheats.

However, in a normal year, good and poor varieties alike have a satisfactory protein content.

Gasoline color values or, more recently, the carotene determination of the whole wheat has been widely used in predicting the color of the macaroni. This may be done on very small samples. Since macaroni color is at present the most important quality consideration, this test, if dependable, would be very useful, but experience in determining the color of a large number of varieties has shown that some samples may be high in yellow coloring matter and bleach out pale when processed into macaroni. Golden Ball is the most striking example of this phenomenon. Apparently this is because the solvent extracts pigments other than carotene, such as xanthophyll, which possibly exhibit different properties in the macaroni process. Thus different ratios between these two pigments would be reflected in differences in the appearance of the macaroni, although the total color extracted might remain constant.

Furthermore, a sample of durum may be high in carotene, but the desirable color may be masked by shades of red or gray, which are extremely undesirable in the production of a quality product. In addition, the bright, intense yellow color usually observed in macaroni made from Mindum or Kubanka—75 is not reflected in carotene readings significantly higher than those of other Kubanka strains producing a paler macaroni. The usefulness of the carotene determination may thus be limited to detecting samples that are too low in yellow pigment.

Thus, the various quality tests now in general use serve as auxiliary methods but do not completely indicate quality as determined in the actual macaroni process. With this in mind an attempt has been made to design a procedure usable as an index of quality where only a limited quantity of grain is available. This process follows closely the essential steps in the commercial manufacture of macaroni.

Milling Technique

The wheat samples were cleaned for milling by the means of a laboratory size dockage machine, scourer, and grain separator equipped with sieves and air blast. Samples of 100 g. were conditioned in two stages, first to 13.0% moisture for 24 hours and then finally tempered by raising the moisture content to 17% one hour prior to milling. The moisture after the final temper is higher than required normally in milling larger samples, but experience has shown that a 100-gram sample is subject to greater moisture loss in grinding.

Processing the samples into semolina is conducted according to the flow sheet shown in Figure 1. Four breaks were employed, two on the No. 16 corrugated roll, and one each on the No. 20 and No. 24 cor-

rugated rolls. The rolls (with a spiral of $\frac{1}{2}$ inch per foot) are run dull to dull and have a differential speed of $2\frac{1}{2}$ to 1. The unpurified semolina included all the material passing through the 34 GG sieve and retained on the 44 GG, 52 GG, and 60 GG sieves, which were bulked together and cleaned free from foreign material (mostly bran particles) on an experimental purifier. The throughs from the 30 GG sieve and sometimes the tailings, especially if the stock contains a fair amount of clean, coarse middlings, are reground, bolted, and purified. The throughs from the No. 44 and No. 40, resulting from the first and second purification, are thoroughly mixed and constitute the finished semolina. A relatively strong flow of air was used on both first and second purification.

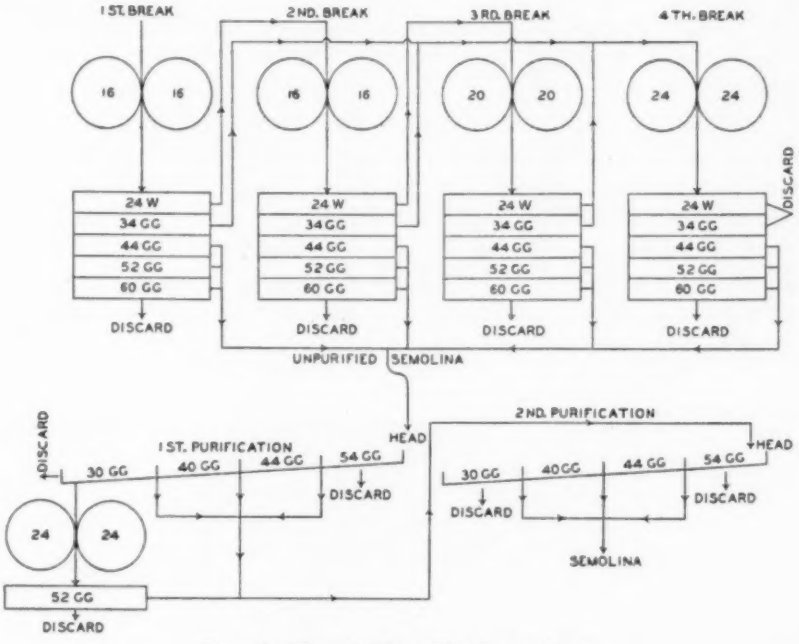

Figure 1. Flow sheet for milling durum wheats.

The milling process described is short, requiring about 20 minutes for the production of semolina from 100 g. of wheat. In durum wheat testing, however, two variables are introduced by the experimental milling process, namely, yield of finished product and granulation. Obviously, yield of semolina from the experimental mill is lower than the percentage obtained in commercial milling, owing largely to the better means of controlling and standardizing the latter equipment. In spite of a lower yield, the percentages are consistent, varying in relation to the test weights, as illustrated in Table II.

TABLE II

MILLING YIELDS OF CLEAN SEMOLINA EXPERIMENTALLY MILLED FROM SMALL SAMPLES OF DURUM WHEATS OF VARIOUS TEST WEIGHTS

Test weight	Yield
Pounds	%
63.0 and above	33.6
60.0 to 62.9	33.2
58.0 to 59.9	28.9
56.0 to 57.9	28.7
54.0 to 55.9	28.5
51.0 to 53.9	27.2

It is believed that samples varying in test weight and milled commercially will have a greater range in percentage of finished semolina than shown in Table II, but unavoidable loss in milling is greater with small-scale equipment. In any event, the relative yield values obtained fall in the proper order. This seems especially significant when it is considered that durum milling is most difficult, for the tempering, grinding, and long purification processes must be rigidly controlled so that a uniform product will be obtained. This is particularly true in order to obtain semolina of the proper granulation. A series of commercially milled samples was compared with the experimentally milled semolina for granulation. The weighed quantity of semolina was sifted for one minute, three sieves being used, as given in Table III, along with the granulation data.

TABLE III

COMPARISON OF GRANULATION FOR COMMERCIALLY AND SMALL EXPERIMENTALLY MILLED SEMOLINA

	Percent of material			
Milling and variety	on 54 XX	on 8 XX	on 11 XX	through 11 XX
Experimentally milled:				
Kubanka (C. I. 1440)	85.6	12.9	.50	0
Kubanka—75	81.7	16.8	.45	0
Mindum	80.0	18.0	.48	0
Commercially milled:				
A	82	16	.66	.34
B	86	12	.67	.33
C	77	20	2.00	0
D	76	21	1.00	0

Examination of the data presented in Table III shows that the experimentally milled and two of the commercially milled samples, numbers A and B, were in good agreement. Samples C and D, commercially milled, were finer, and consequently less material remained on

top of the 54 ××, resulting in a larger percentage on the 8 ×× and 11 ××. It is very likely that the wheat mix from which these samples were milled contained some wheat other than durum and pulverizing easily, formed a greater percentage of finer material.

Processing Technique

The experimental processing of the semolina into macaroni discs consists of five major operations, as follows: Mixing, rolling, resting, pressing, and drying.

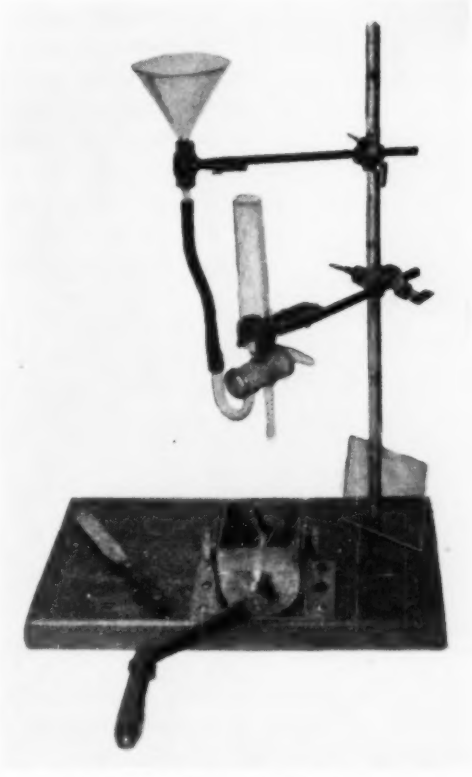

Figure 2. Experimental mixer.

Thirty grams of semolina are mixed with water in a small, especially designed hand mixer, similar in construction to the commercial-type machine. This is illustrated in Figure 2 and consists of a small hopper $2\frac{3}{4}$ inches square and $2\frac{1}{2}$ inches deep, with three pins in the bottom of the trough, which prevent the dough from turning with the mixer arms. There are four mixer arm blades mounted on a shaft which is driven by the means of a handle. The whole unit is clamped in place

by means of a screw device on the sides of the bowl. A small piece of plate glass, 3 inches square, is used to cover the bowl during mixing.

The quantity of water added, to form a dough of proper consistency, approximates 29 to 33%, depending somewhat on the moisture content of the semolina and varying with the seasonal characteristics of the crop.

Mixing continues for one minute, after which the dough is worked by passing between steel rolls. The rolling equipment, miniature in size, shown in Figure 3, consists of two rolls 1 inch in diameter, $3\frac{7}{8}$ inches long, with an adjustment on the side so that the distance between the rolls can be changed. After each rolling, the dough is

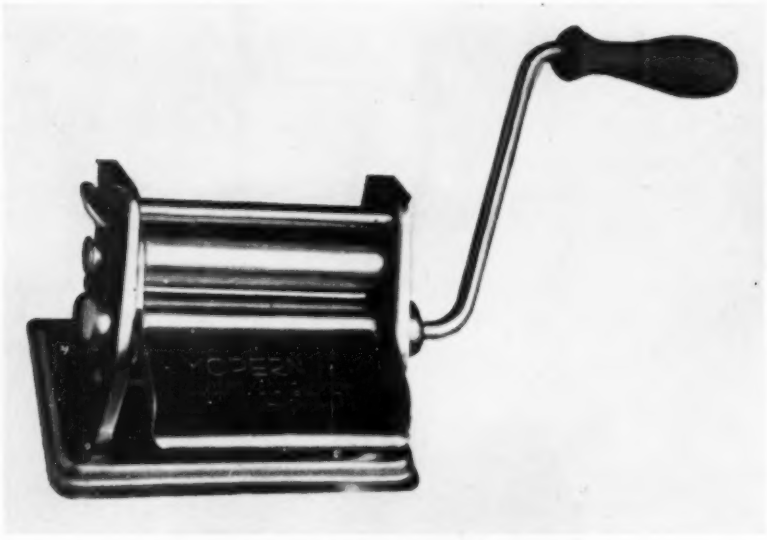

Figure 3. Experimental rolls.

folded lengthwise so that it is repeatedly squeezed and stretched in an action similar to that of the commercial kneading machines. This action increases the plasticity and smoothness of the dough. The procedure was standardized by passing the dough through the rolls 20 times, set at the wide adjustment, measuring $\frac{1}{8}$ inch.

It was found that a smaller number of times through the rolls was not sufficiently severe on the color to bring out the differences observed in the regular experimental macaroni process. For example, with a large number of rolls, the color of Mindum remained clear and yellow, while that of Golden Ball gradually became more white.

After rolling, the dough is formed into a ball and placed in a small beaker for 30 minutes and covered with waxed paper held tightly with

a rubber band to prevent evaporation. In the commercial process, the temperature of the dough during the rest period is considered important. In this test, however, room temperature was used, as experimentation showed that different temperatures varying from 80° F. to 120° F. did not seem to affect the color materially. Length of rest period also did not seem to be very important, provided sufficient pressure was used in pressing; therefore, 30 minutes were taken as a convenient time. Usually about 8 or 10 samples can be mixed and rolled before the first sample is ready for pressing.

After the rest period, the dough is rolled into a sheet of uniform thickness by running it through the rolls set at the $\frac{1}{8}$-inch adjustment and two discs $2\frac{1}{4}$ inches in diameter are cut with a cookie cutter. The discs are then subjected to a pressure of 8,000 pounds per square inch for 30 seconds in a laboratory hydraulic press.

Under the high pressure the dough may stick to the metal piston, making its removal difficult, therefore small cellophane discs are placed between the dough and the metal piston to facilitate the removal of the pressed discs.

The length of time the pressure is applied is important and should be not less than 30 seconds. A longer application of pressure is required if the dough has been mixed slightly dry. Enough pressure must be applied to change the color of the disc from its whitish opaque yellow to a uniformly clear translucent yellow. Beyond this point excessive pressure does not seem to modify the color very much. If the pressure is insufficient, or for too short a time, the color transformation will be incomplete, leaving the disc slightly whitish and granular.

After pressing, the discs are dried between two 1-inch × 8-inch painted boards, padded with a smooth surfaced corrugated paper. The smooth surface of the corrugated paper absorbs some of the moisture but at the same time maintains the proper humidity next to the discs, so they do not check from too rapid or uneven drying. About two days are required for proper drying. It is necessary that the dried discs be flat and not warped to obtain accurate color determinations on the color analyzer. The color readings are made with a disc colorimeter, the readings herein reported being taken on an N–A machine supplied by Wallace and Tiernan Company.

Results from Experimental Macaroni Discs

The usefulness of this method of measuring durum wheat quality, was determined from macaroni discs made from reserve samples of the durum wheat varieties grown in plots at Langdon, North Dakota, in 1934 and 1936. The color data on the discs and on the tubular macaroni previously made from the same wheats are tabulated in Table IV.

In general, the macaroni discs (made on the hydraulic press) were higher in yellow and lower in white than the tubular macaroni made from the same wheat. The percentage of yellow color in the finished product has proved the most satisfactory single index of quality, and the ranking of the varieties for yellow was similar for the two processes. The amount of yellow, particularly in the macaroni discs, showed a close correlation with the commercial ranking of the same varieties, previously presented in Table I.

TABLE IV

COMPARISON OF THE COLOR CHARACTERISTICS OF TUBULAR MACARONI AND MACARONI DISCS MADE FROM DURUM WHEAT VARIETIES GROWN AT THE LANGDON SUBSTATION IN 1934 AND 1936 (N–A DISC COLORIMETER)

Variety and year	Tubular macaroni				Macaroni disc				Commercial rank [1]
	Black	White	Red	Yellow	Black	White	Red	Yellow	
	%	%	%	%	%	%	%	%	
1934									
Mindum	47	14	14	25	41.5	9.0	17.0	32.0	1
Kubanka (C. I. 1440)	44	23	13	20	42.0	12.5	14.5	31.0	2
Kubanka—132	40	24	14	22	43.5	13.5	15.0	28.0	3
Golden Ball	41	26	13	20	41.5	18.5	15.0	25.0	4
Monad	41	23	15	15	54.0	13.5	14.0	18.5	5
Commercial sample	32	22	15	31	—	—	—	—	high
1935									
Commercial sample	36.5	22	14.5	27	44.0	7.0	15.0	34.0	high
1936									
Mindum	41	19	14	26	51.0	9.0	13.0	27.0	1
Kubanka—75	41	21	15	23	49.0	12.0	13.0	26.0	2
Golden Ball	46	22	15	17	53.0	11.0	14.0	22.0	3
Monad	43	21	16	20	58.0	9.0	15.0	18.0	4

[1] Commercial rank taken from Table I.

Where no colorimeter is available, the discs may be compared by holding or placing two or more samples toward a window or so the light passes through the discs. The differences noted can be recorded in terms of yellow, pale yellow, or other descriptive color terms.

These data indicate that the macaroni color characteristics of durum wheat may be accurately determined from only 100 g. of wheat when milled into semolina and processed into a macaroni disc.

Discussion

Very little scientific information is available on the development of color in the macaroni process. From observation of the dough at its various stages, it seems that pressure is the outstanding factor in bringing out the clear, bright color so desirable in macaroni products. The milling process begins with bright, hard, vitreous wheat, very similar in cross-sectional color to that desired in the finished product. The

wheat is tempered and milled, and at no time during the processing does this desirable color return until pressure has been applied to the dough. Adding moisture to the wheat in tempering dulls its translucency. The milled semolina has a dry, whitish yellow color. The freshly wet semolina in the mixer is granular until it is smoothed to a putty color when put in the kneader. After kneading, the dough is more rubbery and plastic but still opaque. The rest period, in which the dough is maintained at a temperature of 100° F. or higher, softens it and brightens the color slightly, but the clear translucent yellow does not return until it has been pressed.

The importance of pressure in making macaroni doubtless was noticed early in the development of a suitable manufacturing procedure. In this study, the macaroni discs pressed at 8,000 pounds per square inch were more translucent than the tubular macaroni made from the same sample. This suggests that commercial macaroni may be made more attractive by subjecting it to higher pressures before pressing through the dies. According to LeClerc (1933),[5] the pressure in commercial practice ranges from 2,500 to 5,000 pounds per square inch. Applying this pressure to the macaroni discs required a longer time before a translucent color was attained. It should be mentioned that broken macaroni sticks are brighter and more translucent in cross-sectional color than on the surfaces, which evidently are somewhat whitened by friction with the die.

In searching for an explanation of the effect of pressure in the macaroni process, a moisture determination on the pressed and unpressed discs was made. The content of the unpressed disc showed a greater moisture loss, averaging nearly 1% more than the pressed disc. Also at the end of one hour in the air oven at 130° C. the pressed discs were golden brown, indicating that caramelization had taken place, while the unpressed discs were still white. This suggests that the starch cells have been broken down by pressure and at least partially hydrolyzed possibly into dextrin. Hydrolysis would account for the lower water loss in the pressed disc.

Besides this chemical effect of pressure, there may be some physical effects involved. Possibly the most probable explanation is that the pressure compacts the dough with a loss of air bubbles into a more homogeneous mass, partially accounting for the different light-transmitting characteristics of the pressed discs. Water distribution is doubtless more uniform if the starch envelope is ruptured by pressure. Rodewald (1894),[6] on the assumption that only the water is reduced in volume when starch swells, has calculated that in fully imbibed starch

[5] LeClerc, J. A. Macaroni products. Cereal Chem. **10**: 383–420 (1933).
[6] Rodewald, H. Über die quelling der stärke. Landw. Vers. Sta. **45**: 201–227 (1894).

granules, the water must be under a pressure of 50,000 pounds per square inch.

This study suggests a number of interesting fields for further work. Quantitative determinations of the degree of hydrolysis in unpressed discs, in macaroni, and in pressed discs from the same semolina might lead to improvement of the macaroni manufacturing process to produce more attractive and more digestible macaroni, and also suggests altogether new food products, possibly of the ready-cooked variety. This latter possibility deserves more attention, particularly in view of the high food value of durum wheat.

The smooth, translucent surface of the macaroni discs suggests that macaroni presses using higher pressures that would stamp out the shapes rather than press them through a die might give a more attractive product if they could be efficiently operated. Experience with the macaroni discs also suggests that with higher pressure the product is less sensitive to changes during drying.

The macaroni disc method was designed to facilitate testing of small samples for color, particularly from the durum wheat breeding nurseries. It may also be useful in other quality tests since it embodies the essential steps of the commercial macaroni process. Tests of swelling, absorption, and ability to retain shape after cooking are some further possibilities. Further investigations of the usefulness and limitations of this test are under way.

Summary

A quality test for small samples of durum wheat is described. Samples of 100 g. of wheat are milled and processed into flat, round macaroni discs which are used to estimate the color characteristics of the sample. The procedure follows closely the essential steps of the commercial process—milling, mixing, rolling or kneading, resting, pressing, and drying.

The milling procedure employed for larger samples has been used with slight modifications. Yields of semolina in relation to test weight of wheat, and granulation percentages comparable to commercially milled semolina, indicate the accuracy of the milling procedure.

A specially designed small mixer, a miniature pair of steel rolls, and a laboratory hydraulic press are used in processing the semolina into macaroni discs. The discs are dried between boards padded with corrugated paper. The disc color is analyzed on a colorimeter and reported in percentages of black, white, red, and yellow.

Pressing the prepared macaroni dough at 8,000 pounds per square inch into a disc changes the visual appearance from a dull white opaque color to a clear, translucent yellow. Color analyses have been made

upon the tubular macaroni and the discs. The discs show a slightly higher percentage of yellow and a lower percentage of white when compared with the tubular macaroni.

Moisture determinations made in an air oven run at 130° C. for one hour showed a 1% greater loss of water from the unpressed disc than from the pressed disc. While the color of an unpressed disc was not affected by drying in an oven for one hour at 130° C., the pressed discs were distinctly caramelized, suggesting partial hydrolysis of the starch into dextrin.

The macaroni disc test herein described was applied to some samples of durum wheat from which tubular macaroni had been made previously. Color analysis of the discs compared favorably with those of the tubular macaroni and the varieties ranked in the same order as their known commercial value.

Previously accepted quality tests such as a visual inspection of the grain, wet and dry slicks of the semolina, protein test, and gasoline color extractions of carotene are important as auxiliary tests of both small and large samples but fail to give a complete measure of the quality of the ultimate macaroni. In macaroni manufacture, pressure apparently is the most important factor for bringing out the clear, translucent color so desirable in the product.

THE QUALITY OF RUST-RESISTANT HARD RED SPRING WHEATS UNDER DEVELOPMENT IN CANADA [1]

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(Read at the Annual Meeting, May 1937)

Beginning with the spring of 1937, two new rust-resistant hard red spring wheat varieties, Renown and Apex, developed in Canada, the former at the Dominion Rust Laboratory, Winnipeg, and the latter at the University of Saskatchewan, will be grown commercially in the susceptible areas of Manitoba and Saskatchewan. The development of these two rust-resistant varieties is the result of a concerted effort begun with the establishment of the Dominion Rust Research Laboratory at Winnipeg in 1924. It is not the purpose of this paper to trace in detail the history of this important undertaking any more than to give a brief sketch of the program under way and to discuss particularly the quality of the material which has been developed during the past twelve years. But before going further the rust-resistant variety Thatcher, developed at the University of Minnesota in co-operation with the United States Department of Agriculture, should be mentioned as one destined to be an important variety in Canada in the next few years at least, a considerable acreage having been grown in 1936 and with possibly upwards of 400,000 bushels available for seeding in 1937.

After the severe epidemic of black stem rust in 1916, the Deputy Minister of Agriculture called a meeting of representatives of the Federal Department, Provincial Governments and Institutions, and invited professional men interested in the problem from the United States. Certain measures of attack were agreed upon which led to further studies of the pathologic nature of the fungus. In the year 1924 more financial aid was given which led to a concentrated effort to link pathologic investigations with a breeding program for the development of suitable varieties to cope effectively with the problem. It should be pointed out here that the danger of rust epidemics could not be eliminated as long as the fungus was prevalent south of the Canadian border because the spores were wind-borne northward as the season advanced. Sulphur dusting, although effective, is uneconomical since too large an area of

[1] Contribution No. 106 of the Cereal Division, Experimental Farms Branch, Department of Agriculture. Published as paper No. 117 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.

the growing wheat would have to be covered at too frequent intervals, at the critical periods of an epidemic. The greatest hope lay in producing by breeding methods suitable varieties of wheat which possessed the ability to resist infection from spores of the fungus.

The Breeding of Rust-Resistant Wheat Varieties

Just prior to the time the Dominion Department of Agriculture decided to undertake an intensive wheat-breeding program for black stem rust resistance, promising breeding material in the form of the selections known as Hope and H–44–24 emerged from the work of E. S. McFadden, of the Agricultural Experiment Station at Brookings, South Dakota, who successfully crossed Marquis with Vernal Emmer in 1915. (See Clark and Bayles, 1935.) The crossed material was grown in bulk at Highmore Substation, South Dakota, where Mr. McFadden was conducting co-operative experiments with the United States Department of Agriculture. It was not until 1923, however, that Mr. McFadden made his selection called Hope on his farm near Webster, South Dakota. Hope and H–44–24, wheats of the *vulgare* type, together with a durum variety, Pentad, constituted the chief varieties used in the breeding programs in Canada to transmit the important characteristic of black stem rust resistance to wheat varieties such as Marquis and Reward, which are considered the acme of quality for wheats of the Manitoba Northern class. The rust-resistant variety, Renown, is the result of a cross between H–44–24 and Reward. It should be remembered, however, that the Canadian workers had the benefit of the splendid work being carried out at the University of Minnesota which resulted in the distribution of Thatcher wheat just three years ago. This variety came from a cross between Marquis × Iumillo and Marquis × Kanred, the former two varieties being the parents of Marquillo and the latter two the parents of Reliance, both of which are known to a limited extent by the milling trade. This double crossed material has been used at the University of Saskatchewan in its breeding work for rust resistance. By bringing into this combination H–44–24 and Marquis, the variety Apex was developed.

Before leaving this reference to the varieties entering into the breeding programs, it may be of interest to mention the nature of the resistance possessed by the parent varieties. Hope, H–44–24, and Pentad have in their genetic make-up what is termed mature plant resistance to black stem rust (*Puccinia graminis tritici*). At Winnipeg these varieties are extremely resistant under field conditions although not entirely so when seedlings are grown in the greenhouse. (See Goulden, Newton, and Brown, 1930.) Moreover, this resistance is transmitted simply, and in

the case of H–44–24 × Marquis it is transmitted as a simple Mendelian dominant. (See Neatby and Goulden, 1930.) The varieties characterized by mature plant resistance have remained resistant to a large number of physiologic forms of *Puccinia graminis tritici*. (See Neatby, 1931.)

While the actual breeding of rust-resistant wheat varieties has been a comparatively easy task, facilitated as it has been by the availability of parent varieties resistant to the disease, the care necessary to insure the development of worthwhile rust-resistant varieties to replace Marquis in those areas subjected to the ravages of black stem rust has been a painstaking and arduous task. It has been necessary not only to select constant varieties measuring up to Marquis in yield, days to mature, strength of straw and other agronomic characteristics, but to select those which measure up to Marquis in quality. This has involved the growing and testing of many hundreds of varieties before picking out certain ones to be advanced to a more comprehensive test in the areas where any new variety would have to compete with the old standard varieties.

The Quality Testing of Rust-Resistant Varieties

The breeding of rust-resistant hybrids in Canada is centered chiefly at the Dominion Rust Laboratory, Winnipeg, Manitoba, at the Dominion Experimental Farm, Brandon, Manitoba, and at the University of Saskatchewan, Saskatoon, Saskatchewan. It was not until 1928 that any of this hybrid material was far enough advanced for milling and baking tests. Preliminary selection was carried out on the basis of agronomic and quality data. In 1931 a beginning was made to test the behaviour of a few of the hybrids at a number of stations but it was not until 1932 that a test was made of a comprehensive series of varieties grown at a number of places in the Canadian West. At this stage, the work branched out into a large co-operative project in that promising strains from each breeding point were pooled into one complete series of approximately thirty varieties and these were grown in plots at a number of points in the three Prairie Provinces, including the Dominion Experimental Stations and the Universities of Saskatchewan and Alberta. The quality test work was conducted by the collaborating cereal chemical laboratories of the National Research Council and the Dominion Department of Agriculture which comprise the Grain Research Committee. The Cereal Division laboratory at Ottawa has conducted the tests on individual samples from each location while the laboratories of the Board of Grain Commissioners, the University of Saskatchewan and the University of Alberta have investigated the quality of certain composited groups of each variety. The set-up then comprises the Committee on

Field Crop Diseases and the Committee on Grain Research, both of which operate under the National Research Council and the Dominion Department of Agriculture. The personnel and facilities of the Dominion Department of Agriculture, the National Research Council, the Board of Grain Commissioners and the Universities of Manitoba, Saskatchewan and Alberta are being used to cover all angles of the problem and the findings of each year are reviewed before embarking on the next season's program.

The Effect of Rust on Yield and Quality

Black stem rust has taken its toll on the wheat yields of western Canada for many years. It has been estimated by Greaney (1933) that the average annual loss for the eight-year period from 1925 to 1932 in yield of wheat in Manitoba and Saskatchewan had a value placed at $35,000,000. With such outbreaks as that which occurred in 1935,

TABLE I

EFFECT OF RUST ON GRADE, WEIGHT PER BUSHEL AND YIELD OF STANDARD VARIETIES IN COMPARISON WITH RUST-RESISTANT VARIETIES IN 1935

Variety	Commercial Grade					
	Winnipeg	Morden	Brandon	Solsgirth	Gilbert Plains	Indian Head
Marquis	Feed	Feed	Feed	Feed	Feed	4 special
Reward	6 special	3°	4°	6 special	3°	2°
Ceres	Feed	6 special	Feed	Feed	Feed	4°
Renown	2°	2°	2°	1°	2°	1°
Apex	5	3°	3°	3°	4°	2°
Thatcher	4°	3°	2°	2°	3°	1°
	Test weight in pounds per imperial bushel					
Marquis	37.5	45.0	43.0	40.4	45.5	54.0
Reward	52.5	57.5	55.5	50.5	57.5	63.0
Ceres	38.0	50.0	46.5	39.2	46.5	56.5
Renown	59.5	60.0	62.0	62.0	62.5	65.5
Apex	55.0	59.0	60.0	60.0	58.5	63.0
Thatcher	56.5	59.5	62.5	60.5	62.0	63.0
	Yield in bushels per acre					
Marquis	1.9	5.5	8.8	1.3	1.7	18.2
Reward	6.5	16.0	19.8	5.0	8.7	28.0
Ceres	3.9	13.6	11.3	2.8	3.0	19.7
Renown	21.2	21.9	37.8	16.9	25.6	44.9
Apex	12.3	17.5	30.2	15.6	18.4	35.9
Thatcher	21.8	32.9	43.5	18.2	26.5	43.8

upwards of $85,000,000 were probably lost to Canada by black stem rust. Not only is the yield often cut but the quality of the wheat is impaired due to the production of underdeveloped, thin kernels of low test weight. This is clearly demonstrated in Table I where comparisons are made between the data from susceptible and resistant varieties grown in test plots at a number of stations in 1935.

The Comparative Quality of Rust-Resistant Wheats with Standard Varieties

In Table II comparisons are made between Marquis, Reward and Ceres and the rust-resistant varieties Renown, Apex and Thatcher for the various quality factors: commercial grade, test weight per bushel, wheat protein, loaf volume and flour colour. The average values for grade are based on numerical values ranging from 0 for Manitoba 1 Hard and increasing in units up to the feed grade which is given 7. A sample grading Manitoba 1 Northern would have a value of 1 and Manitoba 2 Northern a value of 2, etc. The lower values, therefore, indicate the ability of the variety to grade high. The test weight per

TABLE II

A Comparison of 3 Rust-Resistant Varieties with 3 Standard Varieties in Certain Quality Characteristics

Variety	Commercial grade				
	1933	1934	1935	1936	Mean
	9 stations	12 stations	13 stations	12 stations	46 station years
Marquis	.78	2.42	4.7	2.08	2.65
Reward	1.11	1.83	3.3	1.83	2.10
Ceres	.78	1.67	4.46	2.00	2.37
Renown	.89	1.50	2.38	1.83	1.72
Apex	.89	1.83	3.46	2.17	2.20
Thatcher	1.11	2.17	2.92	2.25	2.20
Variety	Test weight in pounds per imperial bushel				
	1933	1934	1935	1936	Mean
	9 stations	12 stations	13 stations	12 stations	46 station years
Marquis	63.8	62.5	54.4	61.2	60.1
Reward	65.3	65.1	61.5	63.3	63.7
Ceres	64.0	63.3	55.5	61.8	60.8
Renown	63.7	63.9	63.0	61.2	62.9
Apex	62.9	62.7	61.2	60.8	61.8
Thatcher	63.0	62.5	61.8	60.3	61.8

TABLE II—*Continued*

Variety	Wheat protein in percentage				
	1933	1934	1935	1936	Mean
	9 stations	12 stations	13 stations	12 stations	46 station years
Marquis	15.3	13.6	14.6	16.5	14.97
Reward	16.3	15.4	15.2	17.8	16.15
Ceres	15.8	14.0	15.0	16.5	15.29
Renown	15.6	14.0	15.1	16.8	15.35
Apex	15.4	14.4	15.4	16.6	15.45
Thatcher	15.5	14.8	14.9	17.5	15.67
Variety	Loaf volume in cubic centimeters (malt phosphate bromate formula)				
	1933	1934	1935 [1]	1936	Mean
	9 stations	12 stations	10 stations	12 stations	33 station years
Marquis	828	794	—	885	836
Reward	886	845	—	954	896
Ceres	807	796	—	887	832
Renown	822	801	852	880	835
Apex	838	783	877	890	837
Thatcher	871	832	876	918	874
Variety	Flour colour (carotene in parts per million)				
	1933	1934	1935 [1]	1936	Mean
	9 stations	12 stations	10 stations	12 stations	33 station years
Marquis	1.78	1.93	—	1.70	1.81
Reward	1.81	1.86	—	1.83	1.84
Ceres	1.85	1.78	—	1.81	1.81
Renown	2.06	2.12	2.50	2.05	2.08
Apex	1.85	1.83	2.30	1.72	1.80
Thatcher	2.02	2.03	2.40	1.91	1.98

[1] Not complete on account of severe damage by rust in the case of Marquis, Reward and Ceres at 5 stations and frost damage for all varieties at 3 stations.

bushel indicates the plumpness of the samples and is related to flour yield. The wheat protein percentages and loaf volume data are an indication of the relative baking strength properties of the flours. The loaf volume data were obtained by the methods described by Larmour, Geddes, and Whiteside (1933) in which a formula is used which includes the regular ingredients of flour, water, yeast, salt and sugar with the additions of .3% of high diastatic malt extract (Lintner 250°) .1% $NH_4H_2PO_4$ and .001% $KBrO_3$. Of all the baking test formulas used, we have found the above to be the best for testing experimentally milled

flour of Canadian hard red spring wheats. The flour colour determinations were based on the methods outlined by Ferrari (1933) with the colorimetric procedure described by Geddes, Binnington, and Whiteside (1934) which give an expression of the quantity of yellow pigments in terms of carotene in parts per million.

Without going into too much detail it will be seen from Table II that Renown had the ability to grade better than all 6 varieties but gave the highest carotene values. Reward stood highest in weight per bushel, wheat protein and loaf volume. Of the 3 rust-resistant varieties, Thatcher gave the highest wheat protein percentage and loaf volume while Apex had the best flour colour. The tests indicate that all three rust-resistant varieties rank high in milling and baking qualities and are infinitely better than Marquis, Ceres and Reward under rust epidemic conditions such as occurred in Manitoba and Southeastern Saskatchewan in 1935.

Table II gives only the data for the individual samples tested for the 4-year period covering 6 varieties, but a very extensive mass of data has been collected by the other collaborating laboratories. As mentioned above, the quality data obtained by each laboratory are reviewed each year and from a consideration of the behaviour of each variety in each district, a joint classification together with a general description of the quality characteristics is prepared. Keeping in mind as an ideal, the quality of Marquis when grown under more or less favourable conditions, Renown has been faulted for being yellower in flour colour and having a tendency toward lower volume. Apex has had a tendency to be lower in weight per bushel and flour yield. Thatcher has a tendency to be lower in weight per bushel, slightly yellower in flour colour, and the appearance of the kernels is less attractive.

Other Rust-Resistant Varieties

Many strains which entered the co-operative series during the last 5 years have been dropped for agronomic or quality faults or both. Some of the more recent contenders have indicated better all-around quality characteristics than any of the three named rust-resistant varieties and will probably be heard from later. In the meantime, some very promising material is coming forward at the Dominion Rust Research Laboratory and at the other breeding points with the object of still further improving the milling and agronomic qualities of rust-resistant varieties. In addition, a new and promising selection of Renown has been made which has excelled the parent variety at Winnipeg in protein, loaf volume and flour colour for the past two years and may,

in the not too distant future, be the foundation stock seed for the Renown variety.

Co-operation of Mill Chemists

The co-operation of cereal chemists attached to milling concerns or institutions in Canada, in the United States and in Europe has been solicited and for the past two crop seasons samples of wheat and flour have been supplied of the newer rust-resistant varieties along with those from the old standard varieties. This procedure has a twofold value: It gives us an idea of what the ultimate buyer of our wheat looks for in Canadian wheat and may uncover some point of quality which we may have overlooked. In addition, it helps to keep the probable ultimate consumer in touch with any varietal changes which may take place in Canada.

Summary

The three rust-resistant varieties of hard red spring wheat, Thatcher, Renown and Apex, which have recently become available to the growers of western Canada, have been discussed from the point of milling quality from experimental tests conducted in Canada. The data so far accumulated have indicated that these varieties compare favourably with the standard varieties under normal conditions for the production of good milling wheat of the Manitoba Northern type and are distinctly superior under rust epidemic conditions. A brief outline of the historical background, the breeding program and the methods for evaluating quality have been given. While a good beginning has been made in alleviating future distress due to epidemics of black stem rust in Canada, there is every hope that even more outstanding varieties will become available in the future not only in agronomic characteristics but in quality characteristics as well.

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ON THE FACTORS DETERMINING RICKETS IN RATS FED ON CEREAL DIETS

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The deposition of calcium phosphate in bones is now known to be dependent on many factors, such as the intake and secretion of calcium and phosphorus, the excess of phosphates or alkali in the diet, incomplete absorption of fats and lack of vitamin D which interferes with fat absorption, and absorption of protein; parathormone and phosphatases also play important parts. Some of these factors are of importance in the etiology of rickets, more especially those which give rise to a deficiency of calcium in the serum.

To these Mellanby (1925) has added one which is of much importance to man who largely subsists on a cereal diet. Mellanby maintains that cereals, especially oatmeal and wheat germ, contain a substance or property which promotes rickets. The nature of this property is unknown except that it can be counteracted by an adequate amount of vitamin D in the diet.

The authors have attempted to demonstrate this rickets-producing substance in cereals as a preliminary to its further study, but as will be seen, have failed to detect it.

The alternative explanation of Bruce and Callow (1934) for the rachitogenic effect of cereals is likewise considered. Their explanation is based on the fact that the portion (approximately half) of the phosphorus present in cereals, which is combined in phytin, is not available, as confirmed by McCance and Widdowson (1935), and, therefore, cereals produce rickets by an insufficiency of phosphorus in the diet.

The experiments herein reported consisted of short-time (about five-weeks') feeding experiments, using rats, in which the amount of

rickets was estimated by examination of the bones stained with silver nitrate. Projection drawings of the stained bones were made and finally classified into four categories—(1) severe rickets (+++), (2) rickets (++), (3) slight rickets (+), and (4) normal bones (0)—without, of course, having any knowledge during classification of the various diets given. A summary of the experimental data is given in Table I. In every case chemical estimations of the calcium and phosphorus contents of the diets were made.

Each experiment has in the first column a reference number which is made use of in the text. In each experiment three rats of about 80 g. were used. Growth during the experiment is the average increase or decrease in weight in grams. The remaining columns require no explanation. The germ flour used was a mixture consisting of 75% of white flour and 25% of wheat germ. Hydrolyzed oatmeal was prepared by boiling oatmeal with 2% hydrochloric acid until all the starch had been converted into sugar (3 to 4 hours). The excess acid was neutralized in one case with calcium carbonate and in the other with sodium carbonate. Care was taken to ensure that both calcium lactate and casein were free of vitamin D. In No. 38 the proportion of maize meal to gluten flour was 76 to 20; in No. 39 the proportion of the potato starch to gluten flour was 76 to 20; and in No. 40 and No. 41 the proportions of the potato starch to casein were 76 to 23 and 76 to 20, respectively. In all experiments the daily ration was 10 g. per rat, made into a paste with a little water. Water was also given separately.

The experimental conditions are given in full detail in Table I and in the explanatory paragraph above, so that one can at once consider the results obtained.

In the first place it is evident that when the animals were fed upon pure cereals with or without marmite they invariably developed severe rickets. This is seen to be the case with white flour (No. 1), germ flour (No. 13), germ (No. 14), oatmeal (Nos. 22, 23 and 24), and maize meal (Nos. 33 and 34).

It is to be noted that in all these cases the calcium content of the diet is very low in comparison with the phosphorus content, from 1 : 4.3 white flour to 1 : 12 for pure germ. In order, therefore, to demonstrate any special rachitogenic property of cereals, it is necessary to rectify this disproportion, since it is well known that this itself greatly favours the occurrence of rickets.

This was done by adding a salt of calcium so as to bring the ratio of Ca : P near to 1 : 0.5. The results show that then rickets does not develop. (See Table I, Nos. 2, 3, 4 and 5 for white flour, Nos. 15 and 16 for germ flour, Nos. 26, 27, 28 and 29 for oatmeal, and Nos. 36 and 37 for maize meal.)

TABLE I

Reference number	Cereal ingredient	Dietary composition							Ca : P Ratio	Period on experimental diet	Growth—Average weight change	Bone condition at end of test
		Marmite (brewers yeast extract) per rat	C.L.O. per rat	Calcium			Sodium					
				Lactate	Phosphate $Ca_3(PO_4)_2$	Carbonate	Chloride	Carbonate				
		G.		%	%	%	%	%		*Days*	*Gms.*	
1	White flour	0.25					1.00		1 : 4.3	30	+ 2	+++
2	White flour	0.25		0.50	2.50				1 : 0.5	92	+47	0
3	White flour	0.25		0.50	2.50				1 : 0.5	89	+32	0
4	White flour	1.0		0.50	2.50		1.0		1 : 0.5	35	+50	0
5	White flour	0.25		0.50	2.50				1 : 0.5	35	+40	0
6	White flour	0.25		3.00					4.9 : 1	35	+37	+++
7	White flour	1.0		3.00			1.0		4.9 : 1	31	+60	++
8	White flour					3.00	1.0		10 : 1	28	− 3	+++
9	White flour	0.25				3.00	1.0		10 : 1	23	− 3	+++
10	White flour					3.00	1.0		10 : 1	25	+23	+++
11	White flour	0.25				3.00	1.0		10 : 1	35	+27	+++
12	White flour	0.25	5 drops			3.00	1.0		10 : 1	35	+23	0
13	Germ flour						1.50		1 : 9	30	+17	+++
14	Germ						1.0		1 : 12	21	+15	+++
15	Germ flour			3.00			1.50		1 : 0.45	35	+73	0
16	Germ flour			3.00			1.50		1 : 0.45	31	+70	0
17	Germ flour					3.00	1.50		6.7 : 1	35	+13	++
18	Germ flour	0.25				3.00	1.50		6.7 : 1	35	+33	+++
19	Germ flour					3.00	1.50		6.7 : 1	35	+57	+++
20	Germ flour	0.25				3.00	1.50		6.7 : 1	35	+60	+++
21	Germ flour	0.25	5 drops			3.00	1.50		6.7 : 1	35	+37	0
22	Oatmeal								1 : 5.7	35	+12	++
23	Oatmeal	1.0							1 : 5.7	26	+15	++
24	Oatmeal	0.25							1 : 5.7	30	+25	+++
25	Oatmeal	1.0	5 drops						1 : 5.7	35	+57	0
26	Oatmeal			0.50	2.50				1 : 0.5	35	+10	0
27	Oatmeal			3.25					1 : 0.6	35	+50	0
28	Oatmeal			3.25			1.00		1 : 0.6	31	+53	0
29	Oatmeal			1.63					1 : 1.1	35	+41	0
30	Hydrolyzed oatmeal	1.0						To neutralize HCl	1 : 4.0	35	+12	++
31	Hydrolyzed oatmeal	1.0				To neutralize HCl			5.4 : 1	35	− 7	+++
32	Mixture of 30 and 31					To neutralize HCl		To neutralize HCl	1 : 0.6	28	+35	0
33	Maize meal								1 : 5.9	24	+ 7	+++
34	Maize meal	1.0							1 : 5.9	35	+10	+++
35	Maize meal	1.0	5 drops						1 : 5.9	35	+17	0
36	Maize meal			0.50	2.50				1 : 1.2	35	+25	0
37	Maize meal	1.0		4.00			1.00		1 : 0.44	31	+52	0
38	Maize meal + gluten flour					3.00	1.00		4.9 : 1	35	+30	+++
39	Potato starch + gluten flour	1.0				3.00	1.00		11.4 : 1	42	+42	++
40	Potato starch + casein	1.0					1.00		1 : 1.9	14	+30	++
41	Potato starch + casein			3.00			1.00		1 : 0.38	37	+30	0

It is thus seen that the rickets-producing power of these cereals can be entirely counteracted by adding calcium to the diet. It can also, of course, be destroyed by the addition of vitamin D as, for example, in No. 25, oatmeal, and No. 35, maize meal. If a disproportion of the calcium-phosphorus ratio be the cause of the rachitogenic action of cereals, then it should be possible not only to counteract this

by the addition of calcium, but also by further additions of calcium to make the cereal diet again rickets-producing, in this case due to an excess of calcium. This was carried out in Nos. 6, 7, 8, 9, 10 and 11 with white flour, in Nos. 17, 18, 19 and 20 with germ flour and in No. 38 with maize meal. In all these cases rickets developed.

It was then shown that the appearance of rickets following an excess of calcium could be counteracted by giving vitamin D both with white flour (No. 12) and germ flour (No. 21).

Experiments using potato starch, gluten flour and casein were next made because presumably they do not contain the special rachitogenic factor postulated by Mellanby (1925), in order to see whether by alteration of the calcium-phosphorus ratio potato starch would behave like cereals. First, calcium carbonate was added so as to bring the ratio to 11 : 1 (No. 39). In this case rickets developed. Without the addition of calcium carbonate the ratio was 1 : 1.9 (No. 40), and here also an excess of phosphorus gave rise to rickets. When, however, calcium lactate was added in order to bring the ratio to 1 : 0.38 (No. 41), no rickets occurred.

Bruce and Callow (1934), taking into account the fact that about half the phosphorus in cereals is included in phytin, which is not digested, draw the conclusion from their feeding experiments that the rachitogenic effect of cereals is really due to phosphorus deficiency. The authors' experiments already show that this is not the case since the addition of calcium, to bring the calcium-phosphorus ratio to 1 : 0.5, counteracts the occurrence of rickets. However, to make more certain of this, oatmeal was boiled with 2% hydrochloric acid so as to convert all the phosphorus into uncombined phosphoric acid. In one case, No. 30, this was neutralized with sodium carbonate and the whole then made into a diet now very rich in phosphorus in a digestible form and poor in calcium. In this case rickets developed. In another case, No. 31, calcium carbonate was used for neutralization, resulting in a diet having a high calcium-phosphorus ratio; this also produced rickets. When, however, a mixture of these two preparations was made in such proportions as to bring the ratio to 1 : 0.6 (No. 32), normal bones were formed and no rickets developed.

Conclusions

The conclusion to be drawn from these experiments is that no rachitogenic property of cereals was found other than a high phosphorus-calcium ratio. Even if 50% of the phosphorus in cereals was in the form of phytin and not digestible, there would still be a sufficient excess of phosphorus easily to account for the rachitogenic action of a cereal diet in which this disproportion was not rectified.

The rachitogenic action of an unbalanced Ca : P ratio has been repeatedly shown to apply to children and to domestic animals as well as experimental animals, such as the rat. In this connection the reader is referred to the work of Orr, Holt, Wilkins, and Boone (1924) and Howland and Kramer (1922).

If, as these experiments suggest, the simple addition of calcium entirely counteracts the rachitogenic effect of a diet rich in cereals, then the value of cereals as an article of diet becomes much greater than recent recommendations of the League of Nations and other advisory bodies indicate. At any rate, these results show that further investigations are required before recommendations to the general public in respect to bread are justified.

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A COMPARISON OF SEVERAL METHODS FOR THE DETERMINATION OF THE NEUTRALIZING VALUE OF MONOCALCIUM PHOSPHATE

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The determination of the neutralizing value of monocalcium phosphate has been the subject of numerous investigations and publications which, unfortunately, have never led to a definite and generally accepted method. At the present time a number of methods are practiced which may yield results varying over a range of as much as eight units with the same sample of phosphate. The writers believe a comparison of the more commonly employed methods may help to untangle the present confusion and contribute something toward a general agreement on a standard procedure yielding uniform results within reasonable limits.

Practically all methods for determining the neutralizing value of monoclacium phosphate which have been suggested or are now being practiced are titration procedures with various modifications. The history of this development has been repeatedly reviewed by Herd (1927), and by Adler and Barber (1925), and the merits and deficiencies of the different methods discussed by such writers as Herd (1927), Adler and Barber (1925), and Bailey (1925, 1925a).

The difficulties which are inherent in the methods tried so far may be summarized as follows: The reaction between monocalcium phosphate, sodium hydroxide or sodium bicarbonate consists of the formation of various phosphates which subsequently reenter the reaction thus making it complicated and subject to very slight variations of conditions apparently beyond the control of the chemist. Boiling the monocalcium phosphate with an excess of sodium hydroxide and back titration of this excess while hot to a certain pH value appears to be the only way of obtaining fairly reproducible results. However, as the indicator changes in color extremely slowly and the color to some extent is absorbed by the semi-gelatinous precipitate formed during the procedure, it is almost impossible to perceive the true end-point of the titration. While one analyst may be able to obtain fairly reproducible results, collaborative tests have shown extremely wide discrepancies between the results obtained by different chemists (Bailey, 1922, 1923, 1924, 1925a).

All titration methods based upon boiling monocalcium phosphate with an excess of caustic have been criticized for failing to duplicate baking conditions. True as this is, it should be remembered that the extent to which monocalcium phosphate reacts with sodium bicarbonate during baking and the pH value of the finished product may depend on varying factors such as temperature, buffering action of the flour, and the presence of other ingredients such as milk, eggs, etc. In other words, baking conditions are not always the same. The writers agree entirely with Adler and Barber (1925) who pointed out that the most that may be expected is an analytical method which gives a reproducible answer as to the total acid strength of a monocalcium phosphate sample. To utilize the result in baking should be a matter of experience and knowledge of baking formulae and baking processes.

Experimental

Five samples of monocalcium phosphate covering a wide range of neutralizing strength were selected and their neutralizing values determined in three different laboratories. Sample No. 1 was a crystallized phosphate containing a small amount of free phosphoric acid. No. 2 was a pure crystallized phosphate. Nos. 3, 4 and 5 were plain commercial products made by three different processes.

The methods employed were:

1. Bailey's method.
2. The tentative method of the A.O.A.C.
3. The hot inverse titration method.
4. A modified titration method proposed by the writers.
5. A "barium chloride method."

Bailey's Method [1]

To 26.73 g. of sodium bicarbonate add 41.77 g. of the monocalcium phosphate to be tested. (This assumes a neutralizing value of approximately 64.) Also add, if desired, 31.5 g. of neutral starch and mix thoroughly. Keep dry until used. Add 1 g. of this test baking powder to 100 c.c. of distilled water in a 250 c.c. pyrex beaker. Boil until free from CO_2. When cool determine the pH of the supernatant liquid electrometrically or colorimetrically, using appropriate indicators. Mixtures with different ratios are to be prepared until a pH of 7.0 is obtained.

The procedure was somewhat simplified by establishing first a titration curve for sample No. 3. The neutralizing values of the remaining samples were determined by arranging the ratio of sodium bicarbonate to monocalcium phosphate until a pH close to 7 was

[1] See Bailey, 1928.

obtained. The proper ratio for pH 7.0 was then estimated from the established curve. The mixtures were boiled for exactly 10 minutes. The pH determinations were made electrometrically using a Beckman pH Meter (glass electrode). The method was carried out by one observer only.

TABLE I

NEUTRALIZING VALUE BY BAILEY'S METHOD

Sample number	Parts of sodium bicarbonate per 100 parts of phosphate	pH of supernatant liquid				Average	Estimated neutralizing value at pH 7.00
1	73	6.96	7.00			6.98	73.5
2	70	6.92	6.95			6.935	71.7
	65	6.87	6.86			6.865	
	68	6.98	7.00			6.99	
	70	7.11	7.06	7.01	7.05	7.06	
	75	7.35	7.20			7.275	
3	80	7.60	7.66	7.52	7.38	7.54	
	82	7.52	7.43	7.38	7.52	7.46	
	85	7.60	7.50	7.46	7.67	7.56	
	90	7.61	7.83			7.72	68.4
4	69	7.00	7.02			7.01	68.8
5	65	6.98	6.96			6.97	65.8

Bailey's procedure duplicates baking conditions fairly well but has been criticized for fixing the neutralization point of monocalcium phosphate and sodium bicarbonate at pH 7.0 whereas the pH of the end-products of the reaction is considerably higher (Herd, 1927, and Adler and Barber, 1925). The values obtained are naturally far lower than those of any other method and hardly correspond to values at which baking powders are properly compounded. The difficulties in obtaining reliable and reproducible results are considerable and increase steadily as the pH of the end-products of the reaction is shifted towards the alkaline side.

It should also be mentioned that the use of electrometric equipment seems to be essential. Colorimetric pH determinations will hardly be accurate enough as in the above procedure only a range of approximately three parts of sodium bicarbonate per one hundred parts of monocalcium phosphate can be distinguished.

Tentative Method of the A.O.A.C.

Weigh 0.84 g. of monocalcium phosphate into a small beaker or casserole, add 25 c.c. of water and 3 drops of a 0.2% solution of phenolphthalein, and titrate with 0.2 N NaOH to a faint pink. Heat to boiling, boil 1 minute, and again continue the titration, while the

solution is hot to a faint pink color, adding the bulk of the alkali solution rapidly and with vigorous stirring. The total burette reading multiplied by 2 equals the neutralizing strength of 100 parts of phosphate in terms of $NaHCO_3$. The reaction in this procedure may be expressed by the following equation:

$$3CaH_4P_2O_8 + 8NaOH \rightarrow Ca_3P_2O_8 + 2Na_4H_2P_2O_8 + 8H_2O$$
$$3(252) = 756 \qquad 8(84) = 672$$
$$\frac{672}{756} = 88.9 \text{ parts } NaHCO_3/100 \text{ parts phosphate}$$

The values obtained with this method as listed in Table II show

TABLE II

NEUTRALIZING VALUE BY THE TENTATIVE A.O.A.C. METHOD

Sample number	Collaborator A			B	C		
			Average				Average
1	84.2	83.6	83.9	83.0	85.4	84.6	85.0
2	82.8	82.3	82.55	81.2	85.2	85.0	85.1
3	79.0	79.8	79.4	76.0	79.8	80.2	80.0
4	76.4	77.6	77.0	78.8	78.4	78.4	78.4
5	75.8	75.2	75.5	74.6	75.2	76.0	75.6

rather poor duplicate readings while the agreement between the observers is comparatively close. All the observers had difficulty in obtaining a distinct end-point to the titration.

Hot Inverse Titration Method

Weigh 0.84 g. of the phosphate and place into a casserole. Add 25 c.c. of cold water and after stirring for a moment add exactly 90 c.c. 0.1 N NaOH. Bring the solution to a boil in two minutes and boil for exactly one minute. While the solution is still boiling hot, add 0.05 c.c. of 0.25% phenolphthalein solution and then 0.2 N HCl until the pink color has all but disappeared. Boil the solution again for exactly one minute and add at once standard HCl until the pink color has just disappeared.

The total number of cubic centimeters of 0.2 HCl multiplied by 2 is subtracted from 90 to obtain the neutralizing value of 100 parts of phosphate in terms of sodium bicarbonate.

The reaction in this procedure should theoretically be the same as in the tentative method of the A.O.A.C. The results show, however, that the reaction in the latter case does not proceed as far as in the hot inverse titration method where the calcium is probably completely precipitated as calcium hydroxy-apatite.

Table III shows that observers B and C obtained very satisfactory duplicate readings while observer A was not able to check his results. The actual results of the three observers do not agree at all but show

TABLE III

NEUTRALIZING VALUE BY HOT INVERSE TITRATION METHOD

Sample number	Collaborator									
	A			B			C			
			Average			Average				Average
1	87.55	87.65	87.60	83.30	83.40	83.35	89.8	89.8	—	89.8
2	86.05	87.30	86.60	82.75	82.62	82.68	88.8	88.95	—	88.85
3	84.70	84.15	84.40	80.00	80.08	80.04	87.4	87.5	87.7	87.55
4	84.15	84.15	84.15	79.65	79.75	79.70	86.0	86.1	86.3	86.15
5	79.45	78.20	78.8	77.60	77.50	77.55	83.0	83.0	83.2	83.05

very similar relative strength of the samples. The observers agreed that light conditions greatly influence the reading and that the titration should be made under standardized illumination.

Modified Titration Method (A.A.C.) [2]

The following procedure was developed by the writers in order to avoid errors caused by slight variations of conditions such as boiling time, and light and particle size of the sample, and mainly to obtain a better end-point for the titration:

Pipette 100 c.c. of N/10 NaOH into a volumetric 200 c.c. flask and rinse 0.84 g. of the monocalcium phosphate into the flask with about 10 c.c. of water. Bring the mixture to boil on a medium strong flame within 4 to 5 minutes. Place the flask on an asbestos sheet over a low flame, just strong enough to keep the mixture boiling. Boil for 10 minutes, cool and make up to volume. Filter and pipette 100 c.c. of the filtrate for titration. Add about 30 g. of C.P. NaCl and twenty drops of thymol blue and titrate with 0.1 N HCl to a distinct yellow, using a standard sample for color comparison.

The number of cubic centimeters of 0.1 N HCl multiplied by 2 and subtracted from 100 gives the neutralizing value. The reaction in this procedure is theoretically as follows:

$$10CaH_4P_2O_8 + 28NaOH \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 14Na_2HPO_4 + 26H_2O$$

$$10(252) = 2520 \qquad 28(84) = 2350$$

$$\frac{2350}{2520} = 93.3 \text{ parts } NaHCO_3/100 \text{ parts phosphate}$$

The results in Table IV show sufficiently close duplicate determinations and a fair agreement among the three observers.

[2] American Agricultural Chemical Co.

TABLE IV

NEUTRALIZING VALUE BY THE MODIFIED INVERSE TITRATION METHOD (A. A. C.)

Sample number	Collaborator A			Collaborator B				Collaborator C		
			Average				Average			Average
1	93.3	93.3	93.3	92.80	92.60	92.80	92.73	92.8	92.7	92.75
2	92.6	92.6	92.6	91.48	91.40	91.76	91.55	92.2	92.0	92.1
3	90.1	90.4	90.25	89.40	89.60	89.40	89.50	90.0	90.0	90.0
4	89.6	89.7	89.65	89.42	89.30	89.56	89.42	89.4	89.6	89.5
5	88.2	88.1	88.15	87.40	87.50	87.50	87.49	87.8	87.8	87.8

Barium Chloride Method

In an attempt to avoid the buffering effect of the phosphates formed during the titration of the monocalcium phosphate, the writers tried the following procedure in which the phosphates are eliminated by precipitation with barium chloride:

Pipette 100 c.c. of 0.2 N NaOH into a 200 c.c. volumetric flask and rinse 0.84 g. of the monocalcium phosphate into the flask with about 10 c.c. of water. Bring to boil in 4 to 5 minutes on a medium strong flame and transfer to an asbestos sheet over a low flame, just strong enough to keep the mixture boiling. Boil for 10 minutes, cool and add 10 c.c. of a 20% $BaCl_2$ $2H_2O$ solution. Make up to volume, filter and titrate 50 c.c. of the filtrate immediately with 0.1 N HCl, using thymol blue or phenolphthalein as indicator. The number of cubic centimeters of 0.1 N HCl multiplied by 4 and subtracted from 200 gives the neutralizing value.

The theoretical neutralizing value of a pure monocalcium phosphate was calculated from the following equations:

$$10CaH_4P_2O_8 + 42NaOH \rightarrow Ca_{10}(PO_4)_6 \cdot (OH)_2 + 14Na_3PO_4 + 40H_2O$$

$$6Na_3PO_4 + 10BaCl_2 + 2NaOH \rightarrow Ba_{10}(PO_4)_6 \cdot (OH)_2 + 20NaCl$$

Total number of mols of NaOH to react with 10 phosphate = 42 + 4.66 = 46.6

$$10(252) = 2520 \qquad 46.6(84) = 3920$$

$$\frac{3920}{2520} = 155.5 \text{ parts } NaHCO_3/100 \text{ parts phosphate}$$

As may be seen in Table V, only observer B obtained satisfactorily close readings. While the agreement among the observers is rather poor the relative strength of the five samples is again fairly well demonstrated.

The only advantage of this method was the sharp titration end-

TABLE V

NEUTRALIZING VALUE BY THE BARIUM CHLORIDE METHOD

Sample number	Collaborator A			B			C		
			Average			Average			Average
1	151.5	152.1	151.8	148.20	—	148.20	152.1	152.1	152.1
2	149.5	150.3	149.9	147.60	147.40	147.50	153.0	153.9	153.45
3	146.3	146.7	146.5	144.60	144.72	144.66	147.5	148.3	147.9
4	144.3	144.5	144.4	143.80	143.80	143.80	147.4	149.3	148.35
5	141.1	141.7	141.4	142.40	142.80	142.60	144.9	146.6	145.75

point. In view of the high experimental error, the sharp end-point is of little importance.

The method was therefore believed to have no practical value, but was considered sufficiently interesting to be included in this paper.

Baking Values

In order to compare the actual effect of the neutralizing strength on the pH value of the baked product, biscuits were baked using the five samples of phosphate in the following formula:

	Grams
Flour	100
Hydrogenated shortening	13
Salt	2
Distilled water	56
Phosphate	1.875
Sodium bicarbonate	1.50

The pH values of the finished biscuits based on the averaged results shown in Tables I to V are included in Table VI.

Discussion

Disregarding Bailey's method as not sufficiently practical, we find two methods may be expected to yield reproducible results:

The hot inverse titration method.

The modified titration method proposed by the writers.

Looking over the data in Table VI, it can be seen that in all methods the five phosphates showed a very similar relationship in their acid strength. It is also apparent that the closest agreement between the collaborators was reached with the modified titration method. However, in view of the importance generally attached to the neutralizing value of phosphates, a still closer agreement appears most desirable. It occurred to the writers that by the application of a factor which may vary with the analyst and the method used, the results of either one or both methods could be brought to a very close agreement. In

TABLE VI

COMPARISON OF AVERAGED RESULTS SHOWN IN TABLES I TO V INCLUDING pH VALUES OF BISCUITS BAKED AT RATIO 80 TO 100

Sample number	Collaborator	Bailey	Tentative A.O.A.C.	Hot inverse titration	Modified titration	$BaCl_2$	pH of biscuits
1	A	73.5	83.9	87.6	93.3	151.8	6.90
	B		83.0	83.35	92.73	148.20	
	C		85.0	89.8	92.75	152.10	
2	A	71.7	82.55	86.6	92.6	149.9	7.17
	B		81.2	82.68	91.55	147.5	
	C		85.1	88.85	92.1	153.45	
3	A	68.4	79.4	84.4	90.25	146.5	7.22
	B		76.0	80.04	89.50	144.66	
	C		80.0	87.55	90.0	147.9	
4	A	68.8	77.0	84.15	89.65	144.4	7.27
	B		78.8	79.70	89.42	143.8	
	C		78.4	86.15	89.5	148.35	
5	A	65.8	75.5	78.8	88.15	141.4	7.40
	B		74.6	77.55	87.49	142.6	
	C		75.6	83.05	87.8	145.75	

practice this would mean a general agreement on a definite neutralizing value of a standard sample which might be distributed among interested laboratories. An annual or semi-annual distribution of a check sample, similar to the monthly check-sample distribution in flour analysis, should be helpful in keeping the results of all interested parties in sufficiently close agreement.

Acknowledgment

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A STUDY OF GLUTEN PROTEIN FRACTIONATION FROM SODIUM SALICYLATE SOLUTION. PART I. DURUM WHEAT GLUTEN FRACTIONATION

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Introduction

A summary of the literature dealing with the fractionation of the gluten proteins by treatment with various reagents, such as acids, alkali and alcohol, was published by Harris and Bailey (1937) and will not be included in this article. These researchers applied the method of separation of three gluten proteins described by Blish and Sandstedt (1933) by dispersing washed crude gluten prepared from a number of flours of different characteristics in dilute acetic acid and ethyl alcohol, then precipitating the protein after the addition of a small quantity of potassium sulfate by successively lowering the temperature. What appeared to be three distinct proteins were separated, and the proportions of each correlated with loaf volume. Only one protein, the so-called "gliadin" fraction, proved to be significantly and positively related to loaf volume. As this procedure of protein separation has recently been questioned on the ground that the gluten constituents were more or less denatured by the action of the acid and alcohol involved, it seemed logical to investigate other methods which did not employ acid, alkali or alcohol in dissolving the gluten prior to fractionation.

The action of two neutral solvents, urea and sodium salicylate, upon coagulated proteins was investigated by Hopkins (1930) who found that urea in high concentration exerted a denaturing as well as a dispersive action. He postulated that the denaturation effect appeared to be related to chemical configuration. Anson and Mirsky (1929) found urea and sodium salicylate to alter or denature albumin. These workers (1931) also postulated a double inactivation by alkali, one fast and the other slow. Later Anson and Mirsky (1932) found that the viscosity of protein systems increased during denaturation, and that viscosity was very sensitive to slight changes in salt and hydrogen-ion concentrations. The addition of urea to the point of saturation, however, lowered the viscosity of a viscous solution of egg albumin. Cook and Alsberg (1931) and Cook and Rose (1934) proved that wet crude

gluten washed from wheat flour is capable of being completely dispersed in solutions of urea and sodium salicylate. Later, Rose and Cook (1935) conducted an investigation into the viscosity of gluten dispersed by these two neutral solvents as well as the classical solvents dilute acid and alkali. These workers found, from computations based upon viscosity measurements, that the effective particle size is larger in urea and sodium salicylate solutions. The character of a precipitate obtained upon salting out dispersions in each of these four solvents indicated that the neutral solvents altered the gluten less than acid or alkali. This conclusion received further support from the fact that flours of different quality had essentially the same viscosity when dispersed in acid or alkali, whereas the dispersion in neutral solvents showed a marked variation in viscosity. These variations were found to be partially correlated with baking strength.

The effect of heat treatment on the viscosity of gluten dispersions in the four solvents was next investigated by Cook and Rose (1935) who found that the viscosity of dispersions in alkali and acetic acid invariably decreased during heat treatment, the magnitude of the decrease being much larger in the former solvent. Dispersions in the neutral solvents underwent an increase above 70° C., decreasing later with further rise in temperature, the qualitative difference in the viscosity changes being the only evidence adduced to indicate that dispersed gluten undergoes any sudden change in character at a certain temperature comparable to the so-called " coagulation point " of albumin. These workers later (1935a) postulated that gluten was hydrolyzed even at 0° C. by dilute sodium hydroxide and acetic acid, the reaction being greatest in the former solvent. No appreciable hydrolysis occurred in gluten dispersions in urea or sodium salicylate even following relatively drastic heat treatments. The extent of hydrolysis did not parallel the decrease in viscosity in all cases, however. The authors finally concluded that the neutral solvents, urea and sodium salicylate, are much superior to either alkali or acid as dispersion mediums for gluten.

McCalla and Rose (1935) conducted a research into the fractionation of gluten dispersed in sodium salicylate, using magnesium sulfate in varying quantities as the precipitating agent. Urea was not used since it entailed the laborious procedure of removing the urea nitrogen. These writers found the solubility of gluten in sodium salicylate to increase regularly with increasing concentration of the salt, and that the relation between magnesium sulfate concentration and gluten nitrogen precipitated was constant. Successive fractions were thrown down and later recombined to form a gluten. They thought the most soluble 10–15% of the gluten complex to be distinct but the remainder to be a single protein complex, which can be progressively fractionated.

Mangels and Bailey (1933) treated various starches from durum, winter and hard red spring wheats with different gelatinizing agents, including sodium salicylate. Mangels (1936) examined varietal and seasonal variations in durum wheat starches, while earlier (1934) this worker studied the peptizability of the wheat flour protein using both common and durum wheat as experimental material. The durum wheats as a class tended to show a higher percentage of peptizable protein. In view of this previous work done on durum wheat at this location, and the importance of the durum wheat growing industry in North Dakota, the first part of the following investigation employed durum wheat flour as the experimental material.

The method of protein fractionation by sodium salicylate dispersion of washed crude gluten, followed by fractional precipitation of the dispersed gluten by progressive additions of concentrated $MgSO_4$ solution as outlined, appealed to the author chiefly because of the evidence pointing to lack of denaturation of the protein by this procedure. If the gluten protein could be separated into undenatured fractions the relative proportion of these fractions should probably bear a relationship to the baking strength of the flour, especially in view of the previous experience of the author with other methods of protein fractionation (Harris 1931, 1931a) (Harris and Bailey, *loc. cit.*). Accordingly the following investigation was undertaken to ascertain if the foregoing hypothesis was true.

Experimental

Material and Methods

Glutens were washed from a series of 10 flours, using the 0.1% sodium phosphate solution of pH 6.8 recommended by Dill and Alsberg (1924). The rate of flow was standardized to 65 c.c. per minute, and the washing time at approximately 10 minutes per sample of 20 g. of flour. For each determination 20 g. of flour were doughed up with sufficient sodium salicylate solution to make a stiff dough. The doughs were then allowed to stand under the solution for approximately 30 minutes before washing. Washing was done over a sieve which allowed the starch to pass but retained the particles of gluten. Finally the washed gluten was allowed to rest under the phosphate solution for approximately 30 minutes. The gluten was now pressed dry between the fingers, weighed and recorded as percent of wet crude gluten. Nitrogen determinations were run on the gluten, and the gluten protein of the flour computed therefrom. For the dispersions 4 g. of the wet crude gluten were weighed out and titurated in a mortar with a few cubic centimeters of sodium salicylate solution. The softened gluten was then transferred to a 300 c.c. Erlenmeyer flask which contained 4 g. of gluten

to 200 c.c. of 10% sodium salicylate solution. The flask and contents were vigorously shaken at frequent intervals until dispersion appeared to be complete. The dispersions were then centrifuged and the supernatant liquid decanted from any residual material.

Aliquots of 50 c.c. of dispersion were placed in 125 c.c. Erlenmeyer flasks and the required number of cubic centimeters of $MgSO_4$ solution added slowly with constant stirring. Immediately a white precipitate of protein commenced to form. The flasks were then set aside over night at 0° C. Foaming was prevented by the addition of a few drops of capryl alcohol. The precipitated protein was removed by centrifuging for approximately 20 minutes, washed with 10% sodium salicylate solution containing the same concentration of $MgSO_4$ as the solution in which precipitation occurred and the quantity of protein in the precipitate determined by the modified Kjeldahl-Gunning method. A further definite quantity of $MgSO_4$ solution was added to the liquid and the former procedure repeated. Difficulty in obtaining concordant results became evident as the fractionation process progressed due to several factors; the character of precipitated protein changed causing greater difficulty of separation; errors tended to become cumulative in effect, and the protein particles became smaller and less easy to separate. The volume of the liquid also increased with successive additions of $MgSO_4$ solution. The protein expressed in milligrams precipitated from 100 c.c. of initial dispersion by the successive additions of $MgSO_4$ solution is shown in Table I.

TABLE I

DESCRIPTIVE DATA AND PROTEIN IN MILLIGRAMS PRECIPITATED FROM 100 C.C. OF SODIUM SALICYLATE SOLUTION BY SUCCESSIVE ADDITIONS OF CONCENTRATED $MgSO_4$ SOLUTION

Sample number	Description of wheat	Crude flour protein[1] (N×5.7)	Quantity of $MgSO_4$ solution added in c.c.				
			1	2	3	5	10
		%					
1	Soft Red Winter	8.0	16.0	63.3	87.2	82.1	66.1
9–5	Amber Durum	14.8	9.1	34.8	82.6	100.3	81.5
2–1	Hard Red Spring	15.0	6.8	45.0	90.6	97.4	114.6
9–4	Kubanka (durum)	17.5	5.7	34.8	91.8	88.9	102.6
9–9	Mindum (durum)	18.7	6.8	20.5	70.2	88.3	94.0
10–14	Golden Ball (durum)	16.1	6.3	29.0	71.2	91.2	111.1
10–19	Mindum (durum)	14.2	6.3	24.5	62.7	67.5	119.1
9–10	Mindum (durum)	14.0	6.3	33.6	78.1	67.3	131.1
10–12	Kubanka (durum)	16.1	10.4	54.2	88.9	74.1	134.5
11–23	Durum	15.8	9.1	36.5	83.2	81.5	141.9

[1] 13.5% moisture basis.

A study of the data presented in this table led to the conclusion that successive additions of 3, 8 and 10 c.c. of $MgSO_4$ would be the most

useful for purposes of studying the relationship of the relative protein fractions with other variables, and this would also eliminate to some extent the tedious time-consuming procedure called for by the removal of 5 fractions by reducing the number from 5 to 3. This was the method adopted and used in the remainder of this investigation. The fractions were precipitated, centrifuged, and the total nitrogen determined. As successive protein fractions were removed the dispersions gradually lost their initial turbidity and finally approached closely the appearance of the original salicylate solution.

The flours used in the principal study were 70% patent, experimentally milled from various grades of North Dakota durum wheat of the 1936 crop. These flours were baked by the standard A. A. C. C. method using 2 minutes mix with the Hobart-Swanson and a modification of this method employing a fermentation time of 2 hours instead of the regular 3. To obviate lack of fermentable sugars, 5% of sucrose was used in both baking methods. These flours were of widely different strength, as measured by loaf volume. While the loaf volume of durum wheat flours cannot be considered as an index of the commercial or agronomic value of the wheat in the same respect as in the class of bread wheats themselves, yet in the writer's opinion it does serve to measure in a large degree the relative strength of the gluten, and at the sucrose level used in this instance no lack of gassing power in the doughs was evident. Proteolytic activity, particularly in the 2-hour fermentation procedure, should not be a factor of prime importance.

Discussion

In Table I are shown the varieties and protein content of the wheats from which the flours included in this investigation were milled, and the milligrams of protein thrown down by the quantity of concentrated $MgSO_4$ solution indicated. These data reveal a relatively large proportion of gluten protein precipitated by the smaller initial additions of $MgSO_4$ solution in the case of flour No. 1, followed by a decrease beyond 3 c.c. concentration. This is not true in the instance of the other samples shown. There does not appear to be any regular relationship, however, between quantity of protein precipitated by any concentration and wheat variety or crude flour protein. The data do show quite clearly on the other hand, the increasing quantity of gluten proteins thrown out of solution by successive increases in the volume of $MgSO_4$ solution added. No further addition of $MgSO_4$ was made after the 10 c.c. portion as it was becoming increasingly difficult to obtain concordant results, and from previous work cited as well as from initial work carried out in this laboratory it appeared probable that a further increase in $MgSO_4$ concentration would not materially affect the con-

clusion to be derived from the data already obtained. From the results presented in this table it appeared that if any relationship existed between the relative quantity of the gluten separated by a specified concentration of $MgSO_4$ and gluten quality as registered by loaf volume, it would be shown most probably by using a relatively low concentration of $MgSO_4$. In the initial range the values appeared to be more scattered and showed marked variation among the different flours. Accordingly, 3, 8, and 10 c.c., respectively, were chosen as volumes of $MgSO_4$ solution to be added to the sodium salicylate dispersions in the following work, thus splitting the original soluble gluten into three distinct proteins, a fourth soluble part remaining in solution.

In Table II are presented the name of variety and protein content of the wheat from which the flours used in the research were milled, with

TABLE II

DESCRIPTION OF AND DATA ON WHEATS AND FLOURS USED
(13.5% moisture basis)

Sample number	Variety of wheat	Crude protein (N×5.7)		Flour ash	Wet crude flour gluten	Protein (N×5.7) in gluten	Gluten protein in flour	Loaf volume	
		Wheat	Flour					Short fermentation	Standard fermentation
		%	%	%	%	%	%	*C.c.*	*C.c.*
36– 9– 1	Durum	17.7	16.1	0.876	45.0	25.3	11.4	255	263
2	Durum	17.3	15.7	.714	48.9	26.0	12.7	310	318
3	Mindum	17.8	16.9	.885	55.4	26.4	14.6	372	343
11	Kubanka	19.2	17.9	.838	62.5	26.2	16.4	479	432
12	Mindum	14.2	13.5	.816	42.7	27.4	11.7	432	378
20	Mindum	19.4	17.8	.705	61.3	26.5	16.2	440	406
36–10– 2	Mindum	18.9	18.2	.749	59.1	26.8	15.8	463	428
13	Golden Ball	17.7	16.0	.785	58.2	25.1	14.6	366	365
16	Kubanka	16.6	16.0	.658	54.5	26.0	14.2	534	500
24	Mindum	16.5	15.2	.876	28.6	23.0	6.6	259	280
36– 9– 5	Durum	15.8	14.8	.828	46.8	26.5	12.4	326	282
4	Kubanka	17.9	17.5	1.019	55.0	26.9	14.8	404	358
9	Mindum	19.4	18.7	1.231	54.0	24.8	13.4	267	282
36–10–14	Golden Ball	18.2	16.1	.791	54.8	24.8	13.6	327	329
19	Mindum	15.4	14.2	.862	39.7	25.7	10.2	265	270
36– 9–10	Mindum	15.4	14.0	.705	46.7	26.7	12.5	434	419
36–10–12	Kubanka	16.9	16.1	.774	52.0	28.0	14.6	547	502
36–11–23	Durum	16.6	15.8	.670	50.0	26.2	13.1	500	526
36– 9–21	Mindum	19.9	18.3	.804	60.4	25.4	15.3	316	298
36–10–25	Durum	18.4	17.1	1.589	28.1	22.3	6.3	281	270
36– 9– 6	Durum	18.2	17.7	1.062	54.8	27.7	15.2	295	312
7	Durum	18.2	17.3	.854	55.7	26.4	14.7	356	359
8	Kubanka	19.6	18.1	.908	58.3	26.6	15.5	382	397
14	Kubanka	20.3	19.3	.859	62.3	26.0	16.2	370	340
15	Kubanka	18.5	17.8	.894	57.8	26.4	15.2	414	349
16	Mindum	18.9	17.4	.905	30.0	26.5	7.9	245	259
36–10–11	Kubanka	15.7	15.0	.720	48.3	27.3	13.2	489	422
15	Kubanka	19.5	18.4	.788	62.8	25.7	16.1	476	448
26	Durum	15.0	13.4	.674	39.5	27.8	11.0	354	332
23	Algerian	16.2	15.4	.859	51.2	26.7	13.7	484	448

flour protein, ash, crude wet gluten, protein in gluten, gluten protein present in the flour, and loaf volume by the two baking methods previously described. The range of crude wheat and flour protein is not extremely large, varying from 14.2% to 20.3% for the wheat and from 13.4% to 19.3% for the flours. Thus in so far as this constituent is concerned, all the samples would be ranked as relatively high. However, this high standard of protein quantity was not reflected in satisfactory loaves, as may be judged from a visual inspection of the data. The 20.3% protein wheat yielded loaves in the neighborhood of 355 c.c. by both baking methods, while the 19.4% wheat samples gave loaves of only 267 c.c. by the short method in one instance. Of course, it must be borne in mind that durum flours cannot be ranked and judged in the same manner as *T. vulgare* flours. The gluten was washed without any great difficulty, although samples 36–10–24, 36–10–25 and 36–9–16 were very sticky and tended to disintegrate. Several of the flours gave very low results for gluten protein percentage in the flour due to the extremely small quantity of gluten recovered. Again, it must be remembered that durum wheat gluten is somewhat different in its properties from *T. vulgare* wheat gluten and is more difficult to wash satisfactorily in many cases. The percentage of gluten protein does not appear to be related to the loaf volume, as will be proved later. The protein content of the gluten does not vary greatly, which is what would be expected provided the washing were done in a comparable manner. None of the flours produced very large loaves and many were extremely poor.

The results presented in Table III show the outcome of the fractionation procedure followed. The solubility of the gluten in the sodium salicylate solution appeared to vary from sample to sample, and this value was included in the table, as well as the percentage of the soluble protein precipitated by various additions of $MgSO_4$. Large variations are evident in the results for different concentrations of $MgSO_4$. An effort was made to estimate the concentration of protein remaining in the solution after the separation of the final fraction listed, but this was abandoned due to lack of good agreement between duplicates and extreme variability between samples. The high concentration of salts present caused difficulty in the nitrogen digestion, and several other obstacles arose. Doubtless the determination of the first fraction thrown down is by far the most accurate, comparing favorably in this respect with the determination of wheat or flour protein, and fortunately this was the fraction which proved to be significantly correlated with loaf volume. Considering again the relative proportions of gluten protein removed by the successive additions of $MgSO_4$, it will be noted that the maximum quantity is reached at 8 c.c. At 10 c.c. the quantity has greatly decreased, and further additions would show a gradual decline

TABLE III

PROTEIN IN MILLIGRAMS AS PERCENTAGES OF TOTAL SOLUBLE PROTEIN PRECIPITATED FROM 100 C.C. OF SODIUM SALICYLATE SOLUTION BY SUCCESSIVE ADDITIONS OF $MgSO_4$ SOLUTION

Sample number	Soluble protein mgs. per 100 c.c.	Additions of $MgSO_4$ in c.c.						Total $MgSO_4$ added in c.c.					
		3		8		10		3		11		21	
		mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%
36- 9-1	396	24.4	6.2	176.0	44.4	110.6	27.9	24.4	6.2	200.4	50.6	311.0	78.5
2	422	38.6	9.1	203.4	48.2	125.8	29.8	38.6	9.1	242.0	57.3	367.8	87.1
3	441	44.4	10.1	213.2	48.3	118.6	26.9	44.4	10.1	257.6	58.4	376.2	85.3
11	462	58.2	12.6	225.0	48.7	114.4	24.8	58.2	12.6	283.2	61.3	397.6	86.1
12	464	49.6	10.7	234.8	50.6	116.8	25.2	49.6	10.7	284.4	61.3	401.2	86.5
20	430	38.8	9.0	214.4	49.9	105.6	24.5	38.8	9.0	253.2	58.9	358.8	83.4
36-10- 2	456	46.8	10.3	213.2	46.7	121.4	26.6	46.8	10.3	260.0	57.0	381.4	83.6
13	439	51.2	11.7	187.0	42.6	120.2	27.4	51.2	11.7	238.2	54.2	358.4	81.6
16	464	53.6	11.5	176.6	38.1	103.6	22.3	53.6	11.5	230.2	49.6	333.8	71.9
24	399	26.8	6.7	191.4	48.0	125.8	31.5	26.8	6.7	218.2	54.7	344.0	86.2
36- 9- 5	467	43.9	9.4	183.0	39.2	81.5	17.4	43.9	9.4	226.9	48.6	308.4	66.0
4	473	40.5	8.6	180.7	38.2	102.6	21.7	40.5	8.6	221.2	46.8	323.8	68.4
9	405	27.4	6.8	158.5	39.1	94.0	23.2	27.4	6.8	185.9	45.9	279.9	69.1
36-10-14	427	35.3	8.3	162.4	38.0	111.1	26.0	35.3	8.3	197.7	46.3	308.8	72.3
19	430	30.8	7.2	140.2	32.6	119.1	27.7	30.8	7.2	171.0	39.7	290.1	67.5
36- 9-10	459	39.9	8.7	145.3	31.6	131.1	28.6	39.9	8.7	185.2	40.3	316.3	68.9
36-10-12	493	65.0	13.2	163.0	33.1	134.5	27.3	65.0	13.2	228.0	46.2	362.5	73.5
36-11-23	473	45.6	9.6	164.7	34.8	141.9	30.0	45.6	9.6	210.3	44.5	352.2	74.5
36- 9-21	402	33.6	8.3	194.4	48.3	113.0	28.1	33.6	8.3	228.0	56.7	341.0	84.8
36-10-25	362	25.6	7.1	157.5	43.5	129.8	35.8	25.6	7.1	183.0	50.5	312.8	86.4
36- 9- 6	456	49.6	10.9	222.2	48.7	77.4	17.0	49.6	10.9	271.8	59.6	349.2	76.6
7	470	53.0	11.3	221.0	47.0	84.8	18.0	53.0	11.3	274.0	58.3	358.8	76.3
8	464	53.6	11.5	241.6	52.1	80.2	17.3	53.6	11.5	295.2	63.6	375.4	80.9
14	433	43.2	10.0	216.6	50.0	83.2	19.2	43.2	10.0	259.8	60.0	343.0	79.2
15	456	65.0	14.2	238.8	52.4	86.6	19.0	65.0	14.2	303.8	66.6	390.4	85.6
16	430	41.0	9.5	225.6	52.5	96.8	22.5	41.0	9.5	266.6	62.0	363.4	84.5
36-10-11	479	85.4	17.8	240.6	50.2	85.4	17.8	85.4	17.8	326.0	68.0	411.4	85.9
15	456	58.2	12.8	237.6	52.1	85.4	18.7	58.2	12.8	295.8	64.9	381.2	83.6
26	484	48.4	10.0	234.8	48.5	107.2	22.1	48.4	10.0	283.2	58.5	390.4	80.7
23	478	93.4	19.5	217.6	45.5	88.4	18.5	93.4	19.5	311.0	65.1	399.4	83.5

in protein removed. It would be practically impossible to separate out all the protein dispersed in the sodium salicylate, as noted by Cook and Rose (*loc. cit.*). The values for total $MgSO_4$ added represent the cumulative effect of the additions, and show the total protein removed from the dispersion rather than the rate of protein removal as the $MgSO_4$ is run in.

In Table IV the data are arranged in order of decreasing quantities of gluten protein removed from the dispersion by 3 c.c. additions of concentrated $MgSO_4$ solution. Crude flour protein, crude wet gluten and loaf volume by the two methods of baking are also given. The general trend of decrease in loaf volume with decreasing quantity of protein precipitated is evident.

The correlation coefficients calculated between the various variables are shown in Table V, with corresponding tests of significance. The protein removed by 3 c.c. $MgSO_4$, both as milligrams and as percent of soluble protein, is significantly and positively correlated with loaf volume. The loaf volumes obtained by the two baking methods are very highly related, and the short fermentation procedure was used in this

TABLE IV

COMPARATIVE DATA ARRANGED IN ORDER OF INCREASING QUANTITY OF PROTEIN PRECIPITATED BY 3 C.C. $MgSO_4$ SOLUTION

Sample number	Crude flour protein (N×5.7)	Wet crude gluten	Protein removed by 3 c.c. of concentrated $MgSO_4$ solution		Loaf volume	
			Mg.	Percent of soluble	Short fermentation	Standard fermentation
	%	%			*C.c.*	*C.c.*
36- 9- 1	16.1	45.0	24.4	6.2	255	263
36-10-25	17.1	28.1	25.6	7.1	281	270
24	15.2	28.6	26.8	6.7	259	280
36- 9- 9	18.7	54.0	27.4	6.8	267	282
36-10-19	14.2	39.7	30.8	7.2	265	270
36- 9-21	18.3	60.4	33.6	8.3	316	298
36-10-14	16.1	54.8	35.3	8.3	327	329
36- 9- 2	15.7	48.9	38.6	9.1	310	318
20	17.8	61.3	38.8	9.0	440	406
10	14.0	46.7	39.9	8.7	434	419
4	17.5	55.0	40.5	8.6	404	358
16	17.4	30.0	41.0	9.5	245	259
14	19.3	62.3	43.2	10.0	370	340
5	14.8	46.7	43.9	9.4	326	282
3	16.9	55.4	44.4	10.1	372	343
36-11-23	15.8	50.0	45.6	9.6	500	526
36-10- 2	18.2	59.1	46.8	10.3	463	428
26	13.4	39.5	48.4	10.0	354	332
36- 9-12	13.5	42.7	49.6	10.7	432	378
6	17.7	54.8	49.6	10.9	295	312
36-10-13	16.0	58.2	51.2	11.7	366	365
36- 9- 7	17.3	55.7	53.0	11.3	356	359
36-10-16	16.0	54.5	53.6	11.5	534	500
36- 9- 8	18.1	58.3	53.6	11.5	382	397
11	17.9	62.5	58.2	12.6	479	432
36-10-15	18.4	62.8	58.2	12.8	476	448
12	16.1	52.0	65.0	13.2	547	502
36- 9-15	17.8	57.8	65.0	14.2	414	349
36-10-11	15.0	48.3	85.4	17.8	489	422
23	15.4	51.2	93.4	19.5	484	448

study as a measurement of gluten quality rather than the standard method inasmuch as durum flours appear to ferment more rapidly than common wheat flours. The only other relationship of any practical importance with loaf volume is shown by percent of wet crude gluten. Flour protein and crude gluten were positively correlated, as would be expected.

The combined effects of crude wet gluten and milligrams of protein removed as fraction I on loaf volume was determined, and found to be significantly higher than the effect of either variable alone. In other words, a gain of information relative to gluten strength was secured through a knowledge of the quantity of protein removed as the first fraction.

TABLE V

CORRELATION COEFFICIENTS COMPUTED FROM THE FRACTIONATION AND BAKING DATA

Variables correlated			
X	*Y*	r_{xy}	*P* [1]
Loaf volume (short fermentation)	Loaf volume (standard fermentation)	+.9594	<.0001
Loaf volume (short fermentation)	Crude flour protein	−.0370	>.5534
Loaf volume (short fermentation)	Wet crude gluten	+.4801	.0074
Loaf volume (short fermentation)	Gluten protein in flour %	+.0358	>.5534
Loaf volume (short fermentation)	Protein removed by 3 c.c. $MgSO_4$ (mg.)	+.6985	<.0001
Loaf volume (short fermentation)	Protein removed by 3 c.c. $MgSO_4$ (% of soluble)	+.6620	.0001
Loaf volume (short fermentation)	Protein removed by 8 c.c. $MgSO_4$ (mg.)	+.1984	.2942
Loaf volume (short fermentation)	Protein removed by 8 c.c. $MgSO_4$ (% of soluble)	−.1016	>.5534
Loaf volume (short fermentation)	Protein removed by 10 c.c. $MgSO_4$ (mg.)	+.0757	>.5534
Loaf volume (short fermentation)	Protein removed by 10 c.c. $MgSO_4$ (% of soluble)	−.2101	.4018
Loaf volume (short fermentation)	Protein removed by 11 c.c. $MgSO_4$ (% of soluble)	+.0077	>.5534
Loaf volume (short fermentation)	Wet crude gluten and protein removed by 3 c.c. $MgSO_4$	+.9041	
Protein removed by 3 c.c. $MgSO_4$ (mg.)	Crude flour protein	−.0822	>.5534
Protein removed by 3 c.c. $MgSO_4$ (mg.)	Wet crude gluten	+.3235	.0737
Crude flour protein	Wet crude gluten	+.5631	.0012

[1] *P* = probability of the observed correlation coefficient arising from uncorrelated material through errors of random sampling.

TABLE VI

TESTS OF SIGNIFICANCE OF ADDITIONAL INFORMATION GAINED BY CALCULATING MULTIPLE CORRELATION COEFFICIENTS [1]

Sum of squares		D F	Variance	F	5% Pt.
$1 - (.4801)^2$	.769504	28			
$1 - (.9041)^2$	.182603	27	.006763		
Difference	.586901	1	.586901	86.78	4.21
$1 - (.6985)^2$	.512098	28			
$1 - (.9041)^2$	.182603	27	.006763		
Difference	.329495	1	.329495	48.72	4.21

[1] Methods of Statistical Analysis by C. H. Goulden. Pages 113–114.

The average values for the various determinations are shown in Table VII, the wheats being grouped according to variety. The group classed as durum probably includes both Mindum and Kubanka as well as other varieties. The averages show some marked differences, particularly in regard to the first protein fraction in the instance of Mindum

TABLE VII

VARIETAL VARIATION IN COMPARATIVE AND FRACTIONATION DATA (1936 CROP) [1]

Variety	Crude protein		Ash	Wet crude gluten	Protein fractions [2]			Loaf volume
	Wheat	Flour			I	II	III	
	%	%	%	%				*C. c.*
Mindum	17.6	16.4	0.85	47.8	37.9	193.1	114.2	349
Kubanka	18.2	17.3	0.83	57.0	58.1	213.4	97.3	410
Durum	17.2	16.0	0.91	46.1	41.1	195.3	107.4	335
Golden Ball	17.9	16.0	0.79	56.5	41.0	187.8	116.2	347

[1] Averages of 10 samples of Mindum, 9 of Kubanka, 8 of Durum, and 2 of Golden Ball.
[2] Protein in mg. removed by 3, 8 and 10 c.c. additions respectively of $MgSO_4$ solution.

and Kubanka. This difference is contrary to the findings of Mangels (*loc. cit.*) who discovered no significant variation in protein peptizability or fractionation between these two varieties. The crude flour protein has no influence apparently upon the quantity of protein removed in the first fraction, while the quantity of protein peptized by salt solution and alcohol varies inversely with the protein content of the flour.

Summary and Conclusions

Wet crude gluten was washed from a preliminary set of 10 flours milled from various varieties of wheat, employing a 0.1% sodium phosphate solution of pH approximately 6.8. These glutens were then

dispersed in 10% sodium salicylate solution and fractionally precipitated by the successive additions of 1, 2, 3, 5, and 10 c.c. of concentrated $MgSO_4$ solution. The fractions were removed by centrifuging following each addition. The results obtained showed great variability in quantity of protein separated by the different concentrations of $MgSO_4$ added. A large variation was also evident in the quantity of protein thrown down by the lower $MgSO_4$ concentration and indicated the possible importance of the initial fraction for purposes of gluten quality evaluation.

A more extensive investigation was started using 30 samples of 70% patent experimentally milled flours produced from different varieties of North Dakota durum wheat of the 1936 crop. The washed and dispersed glutens were fractionated by the addition of 3, 8, and 10 c.c. of concentrated $MgSO_4$ solution. The flours were also baked by 2 methods, the standard A. A. C. C. and a modified method using 2 hours' fermentation. A high positive correlation was found to exist between the loaf volumes yielded by these methods.

Crude flour protein was not related to loaf volume, but wet crude gluten was significantly and positively related to both these variables. The quantity of protein removed initially by the addition of 3 c.c. of $MgSO_4$ proved to be correlated with loaf volume, whereas the other fractions did not have a relationship of any great importance with the size of loaf. The quantity of protein in fraction I did not vary with variation in flour protein. The combined relation between the two variables wet crude gluten and protein removed by 3 c.c. $MgSO_4$ with loaf volume was relatively high, and showed a gain of information with the knowledge of protein precipitated as fraction I in addition to the determination of wet crude gluten.

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SOURCES OF ERROR IN THE DETERMINATION OF THE DIASTATIC POWER OF MALT [1]

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Unpublished investigations made by the authors, together with those reported by Coleman (1936, 1937) and Le Clerc (1937), show that large variations occur in Lintner values obtained by different laboratories for the same malt when using the Official Method of the American Society of Brewing Chemists (1936). In these circumstances it seems apparent that the specifications for the method are not sufficiently precise to eliminate major sources of errors. Data supporting this hypothesis have been collected from time to time in this laboratory and are now reported in the belief that they provide information which will be of use in the development of an improved method for the determination of the diastatic power of malt.

The investigations are divided into two sections dealing with: (1) errors which may legitimately be considered as unavoidable under existing official specifications; and (2) errors resulting from the use of various minor modifications which have been suggested in the literature or have come to the authors' attention through correspondence with and visits to other laboratories. In most of the investigations the reducing power of the digested starch solution was determined by the Blish-Sandstedt ferricyanide method which was shown to be suitable for this purpose by Anderson and Sallans (1937). It should be noted, however, that this modification was used only for investigations in which its use would not prevent interpretation of the results in terms of the Official Method.

Unavoidable Sources of Error

Sampling and extraction: Sixteen 155-g. samples were obtained from 2480 g. of a standard commercial malt of 107° Lintner by thoroughly mixing and subdividing it in a Boerner sampler. On each of five days, two of these samples were taken at random for analysis. From one sample, six 25-g. sub-samples were taken and ground separately; the other sample was ground and thoroughly mixed before six 25-g. sub-

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samples were withdrawn. On each day the 12 analytical samples were randomized and the diastatic powers determined by the ferricyanide method. The data, consisting of 30 determinations by each procedure, were subjected to an analysis of variance. The mean square errors for the two procedures, expressed in terms of millilitres of ferricyanide, were 0.0053 and 0.0009.

If it is assumed that the sampling error was completely removed by grinding and mixing before sampling, the value 0.0009 includes only the errors of extraction, diastasis and determination of reducing power. The mean square error of diastases plus determination of reducing power, estimated from 56 pairs of determinations, proved to be 0.0005 in terms of millilitres of ferricyanide reagent, which is equivalent to a standard error for the mean of duplicate determinations of $\pm 0.29°$ Lintner. It follows, by difference $(0.0009 - 0.0005)$, that the mean square error for extraction is 0.0004, equivalent to a standard error for the mean of duplicate determinations of $\pm 0.26°$ Lintner. This figure is based on the assumption made in the first sentence of this paragraph. Actually it is impossible to obtain a reliable estimate of the extraction error, as this cannot be separated from a possible residual sampling error remaining after grinding and mixing carefully.

If it is assumed that the sampling errors were completely removed by the second procedure, then the difference between the mean square errors for the two procedures represents the sampling error. The mean square error for sampling, in terms of millilitres of ferricyanide, is thus 0.0044, which is equivalent to a standard error for the mean of duplicate determinations of $\pm 0.85°$ Lintner. On the other hand, if the possible extraction error is neglected, the mean square error for sampling is 0.0048, equivalent to a standard error of $\pm 0.87°$ Lintner.

It should be noted that the sampling error of approximately $\pm 0.9°$ Lintner was determined for a single malt of average quality. Our experience indicates that for laboratory malts which are evenly assorted and grown the value may be less and that for ragged, unevenly grown grown malts it may be somewhat greater.

The setting of the Seck mill: On each of four days quadruplicate determinations of diastatic power were made on a single sample of average quality commercial malt. The means of 16 determinations at mill settings of 80, 88, and 100 were 89.63°, 89.34°, and 89.19° Lintner. Since official sieving tests indicate that it is highly improbable that differences equivalent to 20 scale divisions in the mill set will occur between laboratories, it may be concluded that the set in the Seck mill is not a source of major errors between laboratories. An investigation of the effect of using different mills, reported in a later section of this paper, indicates that changing the mill setting will have a greater effect

on undermodified malts. It seems probable, however, that the error due to mill setting will not exceed ± 0.3° Lintner.

The substrate and its preparation: Seven commercial soluble starches were used for parallel determinations made with a standard malt. Solutions of the starches were prepared according to the official specifications and duplicate determinations were made with each starch on four successive days.

An analysis of variance showed that the data provided overwhelming evidence that the starches differed in their resistance to enzyme attack. The F value was over 100 times greater than that required for a 1% level of significance, and the necessary difference between means for starches, required for a 5% level of significance, was 0.4° Lintner. The analysis also showed that the mean square due to the interaction between starches and days was less than that due to duplicates. From this it is inferred that minor variations which may occur in the preparation of starch solutions at different times are negligible as a source of error.

A summary of the experimental data is presented in Table I. It should be noted that all samples were bought as Lintner starches, but in the opinion of the authors numbers 4 and 7 were not Lintner starches. It will be observed that only the first three starches gave values which are identical within the accuracy of the test. The range of values obtained, 6.5° Lintner, agrees with that found by Snider and Coleman (1937) for an equal number of starches. These authors, however, considered that the differences they reported did not exceed the experimental errors of their investigation. In spite of this limitation, the two studies provide evidence which suggests that the standard error for starches is of the order of ± 3.0%, which represents the variation permitted by the Official Method.

TABLE I

LINTNER VALUES OF A STANDARD MALT DETERMINED WITH SEVEN COMMERCIAL SOLUBLE STARCHES (MEANS OF 8 DETERMINATIONS)

Starch number	Lintner value uncorrected	Starch correction, ° Lintner	Lintner value corrected
1	107.4	15.8	91.6
2	101.4	9.9	91.5
3	101.1	9.8	91.3
4	91.6	1.5	90.1
5	111.9	22.7	89.2
6	143.2	55.7	87.5
7	139.0	53.9	85.1

The temperature of diastasis: The diastatic power of two malts was determined in duplicate at temperatures of 19°, 20° and 21° C. The temperatures were set to ± 0.05° C. with a standard thermometer and

controlled to ± 0.02° C. with precision regulators. Lintner values of 83.5°, 90.0° and 96.2° even obtained at the respective temperatures for malt number 1, and values of 174.9°, 188.2° and 199.9° for malt number 2. These data show that the temperature coefficient of diastasis is approximately 7% per degree. The variation of ± 0.2° C. in the temperature of the water bath which is permitted by the official specifications is therefore equivalent to ± 1.4% in the Lintner value.

The diastasis (volumetric and timing errors): The mean square error of diastases plus reductions was calculated as 0.00050 from 56 pairs of determinations, and the mean square error of reduction alone was found to be 0.00007 from 64 pairs of determinations. By difference, the mean square error of diastases alone is 0.00043, in terms of millilitres of ferricyanide reagent, which corresponds to a standard error for the mean of duplicate diastases of ± 0.27° Lintner. These values were determined on samples which averaged approximately 100° Lintner.

The determination of reducing power: Our estimate of the standard error of the Official Method of determining the reducing power of the digested starch solution is merely a rough approximation based on general considerations and on the results of several small experiments.

In the Official Method the total time the reaction mixture should boil before the end-point is reached, and the rate at which " almost the whole of the digested starch " should be added to the boiling Fehling's solution, are left almost entirely to the judgment of the analyst. In the more precise specifications of Lane and Eynon (1923) these and other features of the titration are clearly defined.

The effect of differences in the total time of boiling is shown in Table II. The data represent the means of triplicate titrations made on

TABLE II

EFFECT OF BOILING TIME ON RESULTS OF FEHLING'S TITRATIONS

Determination	Boiling time	Titre digested starch	Blank titre	Lintner value
	Minutes	*Ml.*	*Ml.*	*Degrees*
1	2	35.80	32.10	100.2
2	3	35.46	31.73	100.9
3	4	35.25	31.57	101.6

the same solutions by the Lane and Eynon method, the total time of boiling being the only variable. With increase in boiling time there is a decrease in the titre and an increase in the calculated Lintner value. When a uniform timing procedure is used for both the digested starch and blank determination, the variation in results is not large. However, if the timing is not standardized, it is possible that the digested starch

titre for determination 1 might be combined with the blank for determination 3 and *vice versa*, which would give Lintner values of 98.5° and 103.4° respectively. It is therefore apparent that a standard boiling period should be clearly specified and precisely observed.

In order to demonstrate the effect of the rate at which "almost the whole of the digested starch solution" is added to the boiling Fehling's solution, burette tips were made to deliver 36 ml. in approximately 15 and 110 seconds, respectively. The first tip added the solution about as fast as it is possible to do so and the second added it at such a rate that the reaction mixture boiled continuously. In using these tips the total boiling time from the end of the addition of the reducing solution, and the method of adding the solution near the end-point, were standardized by using the Lane and Eynon method.

Data for two experiments are given in Table III. It will be noted that decreasing the rate of addition of almost the whole of the reducing solution increases the titre for the digested starch solution but decreases that of the blank determination. The data are curious, but, since they were reproduced after an interval of two months with a malt of about the same Lintner value, they appear to be accurate. They must result through obscure interaction between the effects of increasing dilution of the reaction mixture and time of heating on the titre.

TABLE III

EFFECT OF RATE OF ADDING THE REDUCING SOLUTION

Rate of addition	Titre in ml.		Mean Lintner value	
	A. Starch	B. Blank	Uncorrected	Corrected
	Experiment No. 1, April 21, 1937			
36 ml. in 15 seconds	36.15	32.70		
	36.22	32.75	110.5	99.9
36 ml. in 110 seconds	36.55	32.30		
	36.60	32.32	109.4	96.6
	Experiment No. 2, June 21, 1937			
36 ml. in 15 seconds	36.80	33.05		
	36.82	33.15	108.7	97.7
36 ml. in 110 seconds	37.15	32.75		
	37.18	32.70	107.6	94.8

The experiments show that a change in the rate of adding the solution has a comparatively small effect on the uncorrected Lintner value. However, when the corrected Lintner values are calculated by multiplying by the blank titre and dividing by the starch titre, according to the official specifications, the difference between the methods of addition is increased to 3%.

In view of the above considerations, and bearing in mind that the end-point of the titration is not particularly sharp and is affected by the

speed at which the solution is added as the end-point is reached, it is apparent that the official specifications are so loosely worded as to permit wide variations between duplicate determinations. In the hands of inexperienced analysts such variations occur frequently. However, experienced analysts invariably standardize their procedures consciously or unconsciously and it therefore seems probable that the standard error for the mean of duplicate determinations of reducing power made within one laboratory is of the order of $\pm$ 1.0° Lintner.

Even when the more precise specifications of Lane and Eynon are followed, considerable practice is necessary before an analyst is able to judge almost the whole of the reducing solution to be added so that not more than 1.0 ml. is needed to complete the titration. This difficulty is due to the different quantities of solution required for the preliminary and final titrations. Furthermore, it is difficult to estimate the correct rate at which the final increment must be added so that the end-point is reached in exactly 3 minutes. In spite of these difficulties, a high degree of precision can undoubtedly be obtained by the Lane and Eynon method. Calculations based on 32 pairs of determinations having a mean value of 140° Lintner yielded a standard error of $\pm$ 0.32° Lintner for the mean of duplicate determinations.

Standardization of procedure and solutions for determining reducing power: It seems probable that experienced analysts using the Official Method standardize their timing procedure and thus obtain reproducible results. On the other hand, it seems improbable that analysts in different laboratories will adopt the same timing procedure and since the official specifications allow great latitude in this respect, a considerable variation arises between laboratories.

Other sources of error also exist between laboratories. It has been pointed out by Lane and Eynon (1925) that the copper content of different samples of copper sulphate may differ by as much as $\pm$ 0.7% and that the results of Fehling's titrations may be in error by this amount unless the Fehling's solution is standardized. The official specifications provide for standardization by stating that the Fehling's solution should be checked "against a standard solution of invert sugar according to customary quantitatively analytical procedure." The use of a titrimetric procedure is implied. However, the data presented by Ling and Carter (1930) show that unless the conditions of the titration are rigidly defined the titre of an invert sugar solution containing 2 mg. per millilitre may vary by as much as 7.0%. Even for a standardized method of titration the titre is not directly proportioned to the concentration of the sugar solution. It is therefore evident that the concentration of the invert sugar, the exact conditions of titration, and the correct titre for the given concentration of invert sugar should be specified before an ade-

quate standardization of Fehling's solution can be made. If it is assumed that the standardization is to be made by the Official Method of titration, it is still probable that variations of 2% to 3% in the titre for a given concentration of invert sugar solution may occur.

It might reasonably be expected that, if exactly the same method of titration is used for both the determination and the standardization, any errors resulting from differences in interpretation of the official specifications would be eliminated. This does not necessarily follow, as is shown by the data presented in Table IV. This experiment forms an extension of that reported in the first half of Table III, the Lane and Eynon method has been added, and all results have been "corrected" for the effect of the titration method in accordance with the results obtained by standardizing against an invert sugar solution. It will be observed that, when the Lintner values were corrected for the respective standardization titres, the difference between methods C and D was increased, since smaller titres of invert sugar and larger titres of digested starch were obtained with method D than were obtained with method C. This is the result of differences in the behaviour of invert sugar and maltose toward Fehling's solution. Thus, for instance, Lane and Eynon's tables show that the quantity of invert sugar equivalent to a given volume of Fehling's solution increases with dilution of the reaction mixture, but the relation is reversed for maltose. The experiment demonstrates quite clearly that differences in procedure cannot be compensated for by standardizing against invert sugar solution.

TABLE IV

EFFECT OF DIFFERENT METHODS OF ADDING REDUCING SOLUTIONS TO FEHLING'S SOLUTION

Titration method[1]	Titre in millilitres			Lintner value		
	Invert sugar 1.5 mg./ml.	Digested starch	Blank	Uncorrected	Corrected for blank	Corrected for standardization
A	34.41					
B	34.74	35.63	32.17	112.3	101.4	101.1
C	34.97	36.19	32.73	110.5	99.9	100.3
D	33.66	36.57	32.31	109.4	96.6	93.4

[1] A, Standard Lane and Eynon, 10 ml. Fehling's undiluted; B, Lane and Eynon, 10 ml. Fehling's diluted with 10 ml. of water; C, Official, reducing solution added rapidly to boiling Fehling's; D, Official, reducing solution added so that the reaction liquid boiled continuously during the addition.

These considerations, together with those discussed in the last section, suggest that the standard error for standardization of procedure and solutions in the Official Method may well be of the order of $\pm$ 3.0° Lintner.

Discontinuous procedure: At intervals of 0, 3, 6, and 9 hours, duplicate diastases were prepared from a malt infusion maintained at 20° C. The values obtained were 105.7°, 106.2°, 106.7°, and 106.9° Lintner.

A similar series of determinations of reducing power of digested starch solutions at intervals of 0, 4, 6, and 9.5 hours gave values of 105.7°, 107.5°, 108.2°, and 109.3° Lintner.

These changes are dependent on temperature since a set of control solutions maintained at 0° C. showed no significant changes for periods of 9 hours.

Although the official specifications do not state that the determination of diastatic power must be completed in one day, it seems reasonable to suppose that most chemists adopt this practice. Under these conditions it appears that the time elapsing between the different stages of the determination in different laboratories will not vary greatly and that a standard error of $\pm 0.3°$ Lintner will probably represent this source of error.

Errors Due to Modification of the Official Procedure

Kjeldahl's law: It is customary in some commercial laboratories to determine the complete range of Lintner values on a standard aliquot of starch solution with a standard aliquot of the infusion. This procedure takes no account of Kjeldahl's law and results in errors at high Lintner values, as is shown by the data presented in Table V. A malt

TABLE V

DEVIATIONS FROM KJELDAHL'S LAW OF DIRECT PROPORTIONALITY USING 100 ML. OF BUFFERED STARCH AND 2 ML. OF DILUTED INFUSION

Concentration of infusion	Lintner value		
Ml. diluted to 200 ml.	Calculated	Found	Difference
10	11.1	10.9	0.2
30	33.3	33.3	0.0
50	55.5	55.6	−0.1
70	77.7	77.5	0.2
85	94.4	94.7	−0.3
100	111.0	110.8	0.2
115	127.7	126.5	1.2
130	144.3	140.8	3.5
150	166.5	162.1	4.4
170	188.7	180.1	8.6
190	210.9	194.9	16.0
200	222.0	201.4	21.6

infusion having a diastatic power of 222° Lintner was prepared and dilutions made as shown in the table. Diastases were prepared using 2 ml. of the diluted infusions, 100 ml. of buffered starch solution, and 10 ml. of 0.5 N alkali in 250-ml. flasks. This procedure was used to

permit the determination of values up to 200° Lintner by the use of 10 ml. of ferricyanide solution. The direct proportionality between reducing substances present and found by the ferricyanide method was previously established (Anderson and Sallans, 1937) for the range of values covered in the experiment.

Use of mills other than the Seck mill: Samples of three malts were ground in a Seck mill, a Wiley mill, and a Hobart coffee mill at two different settings. Sieving tests showed that the malts were ground more finely in the Wiley mill and in the coffee mill at one setting, and more coarsely at the other setting, than in the standard Seck mill. Relatively different amounts of material remained on the various sieves for each mill. Triplicate determinations were made on each malt for each mill. Table VI shows the mean values for each malt by each method of grinding. For malt number 2, which was very mealy and well modified, the maximum difference was only 0.7° Lintner, while for malts 1 and 3 the differences were 3.6° and 4.6° Lintner.

TABLE VI

MEAN LINTNER VALUES FOR THREE MALTS GROUND IN FOUR MILLS

Mill	Seck	Wiley	Hobart at 1.5	Hobart at 2.5	Maximum difference
Malt number					
1	95.2	96.1	98.8	97.0	3.6
2	93.7	93.0	93.2	93.2	0.7
3	110.5	110.2	114.1	109.5	4.6

Extraction: Gore and Steele (1935) suggest that extraction of the ground malt for 1.5 hours at room temperature is adequate for the preparation of the enzyme infusions.

Quadruplicate infusions of one malt digested at 20° C. for periods of 1.5, 2.0, and 2.5 hours gave values of 85.4°, 87.3°, and 89.4° Lintner respectively. Quadruplicate infusions of another malt digested for 2.5 hours at 20° and 25° gave values of 93.0° and 96.5° Lintner respectively. It would appear that standardization of the time and temperature for the digestion is essential if comparable results are to be obtained in different laboratories.

Ratio of malt to water during extraction: In the method used in Germany, described by Pawlowski (1932), smaller samples of malt are used for malts of high diastatic power, rather than smaller aliquots of infusion or larger aliquots of starch. Infusions prepared and filtered at concentrations of 10%, 5%, and 2.5% gave values of 114.3°, 107.1°, and 101.5° Lintner respectively. For results to be comparable, infusions must be prepared at a definite ratio of malt to water.

Filtration: Twelve infusions were prepared from the same malt, four were filtered, four centrifuged, and four were allowed to settle according to the method of the Institute of Brewing (1923). The mean values obtained were 106.8, 106.5, and 104.0° Lintner respectively. The difference between filtering and centrifuging is not significant, but the settling procedure gives significantly lower results than filtration.

Technique of making diastases: The method of pipetting the infusion into the soluble starch was compared with the Official procedure of adding the starch to the infusion. Triplicate determinations made with undiluted infusions gave values of 106.2° and 108.7° Lintner, and with infusions diluted 1 to 5, the values were 106.1° and 108.4° Lintner respectively. The method of adding the infusion to the starch gave values approximately 2.4° lower than the Official Method, and dilution of the infusion made no difference in the relative values obtained.

Discussion

The results of investigations of those errors which may legitimately be considered as unavoidable under existing official specifications are summarized in Table VII. Those errors which affect the reproducibility of results within any one laboratory are listed in the first half of the table and those which specifically affect the reproducibility of results in different laboratories are listed in the second half. The data must

TABLE VII

ERRORS OF THE OFFICIAL METHOD ESTIMATED FOR A MALT OF 100° LINTNER

Sources of error within one laboratory	Standard errors of mean of duplicate determinations
Sampling	± 0.9
Preparation of soluble starch	negligible
Diastasis (volumetric and timing errors)	± 0.3
Discontinuous procedure	± 0.3
Determination of reducing power	± 1.0
Total error within one laboratory	± 1.4
Sources of error between laboratories	**Standard error for a single laboratory**
Mill setting	± 0.3
Selection of soluble starch	± 3.0
Variation in temperature of diastasis	± 1.4
Standardization of solutions and procedure for determining reducing power	± 3.0
Total error between laboratories	± 4.5
Standard error of means of duplicate determinations made in different laboratories	± 4.7

be considered as approximate estimates but probably offer a fairly reliable indication of the comparative importance of the various sources of error.

The data suggest that a reasonable degree of precision can be obtained within one laboratory, and this we believe has been the experience of most chemists. If improvement is necessary, it must be sought by adopting a more rapid and convenient method of determining reducing power, such as the Blish-Sandstedt ferricyanide method. Investigations made with this modification of the Official Method in these laboratories have demonstrated that standard errors for the means of duplicate determinations of ± 1% can be obtained quite readily within one laboratory.

The results of the investigations suggest that the errors which specifically affect the reproducibility of results in different laboratories are relatively large and that the standard error of the mean of duplicate analyses made in different laboratories is of the order of ± 5° for a malt of 100° Lintner. Recent investigations show that this estimate is not grossly in error. Both Coleman's (1937) data for 12 laboratories[2] and Le Clerc's (1937) data for 10 laboratories yield standard errors of ± 6° Lintner. It seems probable that the value ± 6° represents the highest level of precision that can be obtained with existing specifications and that estimates of certain errors made in this paper are too low.

On the other hand it is possible that some laboratories are using minor modifications of the Official Method. A summary of the modifications examined in this laboratory is presented in Table VIII. The data emphasize the dangers of deviating from the procedure specified in the Official Method.

TABLE VIII

ERRORS INTRODUCED BY MODIFICATIONS OF THE OFFICIAL METHOD

	Modification	Error Percent
1	Failure to correct for Kjeldahl's Law (at 200° Lintner)	− 6.0
2	Grinding samples in different mills	± 2.0
3	Extraction for 1.5 hr. at 20° C.	− 4.6
4	Extraction at room temperature (25° C.)	+ 3.7
5	Extraction of 12.5 g. malt with 500 ml. of water	− 5.0
6	Centrifuging digests	negligible
7	Allowing digests to settle and pipetting supernatant infusion	− 1.5
8	Diastasis at 21° C.	+ 7.0
9	Pipetting infusion into soluble starch	− 2.0
10	Neglecting correction for extract blank	+ 2.0 to + 4.0

The authors believe that the weakest part of the Official Method is the determination of reducing power. This is extremely cumbersome,

[2] One value was left out as it was obviously in error.

particularly with respect to the determination of blank corrections. Moreover, it is so sensitive to personal idiosyncrasies of manipulation which cannot be readily corrected for by an auxiliary standardization procedure, that the problem of obtaining strictly reproducible results in different laboratories seems almost insoluble. It seems probable that a considerable improvement could be effected by adopting the Blish-Sandstedt ferricyanide method for determining reducing power, which has been shown to be directly applicable to the determination of Lintner values by Anderson and Sallans (1937).

The situation with respect to starch appears to be that one cannot expect to obtain reproducible results from Lintner starches bought at random. This point is fully recognized in the Official Method. However, it is doubtful whether the existence of detailed specifications for starch is of much use to the routine analyst. What he requires is a reliable source of supply. It is suggested that the starch difficulty may best be overcome by specifying one brand and grade of starch as official. A limited experience in this laboratory indicates that little variation exists between samples of the same grade of starch bought at different times from a reputable company.

In view of the high temperature coefficient of diastasis, it appears that it would be wise to adopt more stringent specifications for the temperature of the water bath. Since standard open-scale thermometers and sealed mercury-in-glass thermoregulators which will control to $\pm 0.02°$ C. are available at reasonable cost, the temperature for the bath might well be set at $20° \pm 0.05°$ C. Even if such precision were not attained in all laboratories, attention would be directed to the importance of making diastasis at the correct temperature.

The authors believe that if changes are made in the specifications for the starch, the temperature of the water bath, and the method of determining reducing power, and if precise and detailed specifications are drawn up for the complete determination, then it should not be difficult to reduce the standard error for the determination to $\pm 2°$ Lintner or less.

Summary

Sources of error in the determination of the diastatic power of malt by the Official Method of the American Society of Brewing Chemists have been investigated. The standard error for the mean of duplicate determinations made in different laboratories is estimated as $\pm 4.7°$ Lintner for a malt of 100° Lintner. The main sources of error are: standardization of procedure and solutions for determining reducing power, $\pm 3°$; selection of soluble starch, $\pm 3°$; setting tolerance for temperature of water bath, $\pm 1.4°$; and sampling, $\pm 1°$ Lintner.

Investigations of a number of minor modifications in the Official Method emphasize the dangers of deviating from the specified procedure.

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MIXING DOUGHS IN VACUUM AND IN THE PRESENCE OF VARIOUS GASES

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The texture of bread is controlled to some extent by the number of gas bubbles present in the dough. It was conceived that bubbles in a dough might be initiated by the air which was emulsed in the dough during the mixing. Therefore, if gas could be prevented from dispersing in the dough during mixing, then each bubble would need to originate independently by forming around each individual yeast cell or group of cells. Thus many more gas cells might be initiated in the dough than would be possible where air had been emulsed to form gas pockets into which the yeast cells could discharge gas. In an effort to investigate this idea a machine was constructed so that doughs could be assembled and mixed under vacuum and then subsequently fermented and baked. For this purpose a Swanson Mixer was mounted on a special base carrying a motor under the base (see Figure 1), so that the mixer could be operated under a bell jar in a vacuum and the dough mixed while under the vacuum, with special arrangements for adding the water, yeast emulsion, and various ingredients, and controlling the temperature of the dough, when the vacuum had been applied to the flour. Upon baking doughs mixed in this manner a very discouraging result was obtained as regards the original idea behind the investigation. Instead of obtaining upon fermentation a very fine textured dough with many minute bubbles at each yeast cell, the opposite was obtained; namely, a dough that gassed freely. The gas collected in large bubbles and for a period of time the dough appeared unable to retain gas. However, such doughs after the first punch became normal in character and made normal bread.

During the investigation some of the doughs were subjected to excessively long mixing periods and still made similar bread. This observation that dough could be mixed in a vacuum for abnormal periods of time without apparent damage to the bread altered the course of the investigation to the subject of this discussion.

It was apparent that there is some action occurring in the ordinary mixing of dough as universally practiced in the presence of air, which is not present in a vacuum dough. Since the technique involved was long and laborious, the amount of bread that can be baked in a single

day was restricted to a maximum of four to six vacuum loaves, so that it was necessary to limit the variables to as few as possible. Hence, no study has been made of changes in dough ingredients, temperature, speed of mixing or absorption. All studies here have been made with but one speed of the machine—52 r.p.m.—which appears to develop most doughs in from three to five minutes. The only variables studied have been the duration of mixing and the nature of the gas in contact with the dough. The amount of sugar used in the formula has been high enough throughout to eliminate any effect upon the loaf from sugar starvation of the yeast. All loaves have been proofed to the same volume, which has always taken approximately the same length of time.

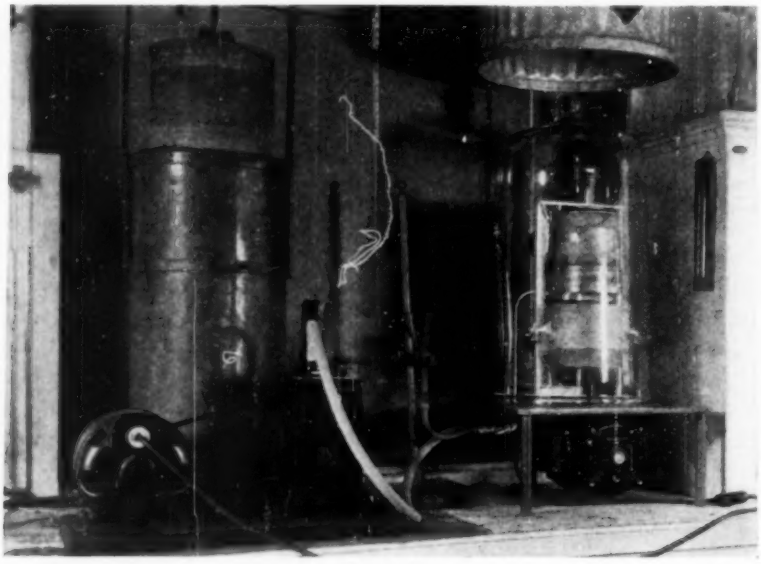

Figure 1.

The first study made was the relationship of mixing to emulsification of gas. The amount of gas emulsed in a dough was indicated by measuring the density of the dough at any given period of mixing. The densities of the dough are determined by placing a weighed amount of dough in a calibrated cylinder containing mineral oil and noting the volume of the dough. The amount of gas emulsed can be calculated from the formula:

> Density of vacuum dough minus density of gas-containing dough divided by density of vacuum dough equals (when multiplied by 100) the percentage by volume of gas emulsed.

The amounts of gas emulsed have not been calculated.

A vacuum dough gives densities of approximately one and one-quarter times the density of water. It is possible by thorough mixing and emulsification, under the conditions of our experiments, to produce doughs with a density as low as 1. Hence 20% of the volume of the dough may be emulsed gas. The dough has increased its volume sufficiently to take up this amount of gas.

Table I records the density of doughs when mixed with various gases for varying lengths of time.

TABLE I

EMULSIFICATION OF GASES DURING MIXING OF BREAD DOUGHS; DENSITY AND DOUGH CHARACTER AT VARIOUS TIMES OF MIXING

Gas	3 Minutes	5 Minutes	20 Minutes	40 Minutes
Air no yeast	1.16 normal	1.13 normal	1.04 stringy	1.07 short
Air + yeast	1.15 normal	1.11 normal	1.02 stringy	1.03 short
Hydrogen + yeast		1.20 normal	1.08 soft	1.03 very soft, sticky
Nitrogen + yeast		1.11 normal	1.08 sticky	1.04 soft, sticky
Oxygen + yeast	1.20 normal	1.16 sticky	1.15 very short	1.15 very short
Vacuum + yeast		1.21 dense, normal	1.23 dense, smooth	1.24 dense, smooth

It is to be noted that when air is mixed in a dough containing no yeast, emulsification of gas proceeds as mixing progresses, followed by some apparent loss of gas at the end of 40 minutes of mixing. When yeast is present the dough performs in a similar manner except that no gas is lost after continued long mixing, probably due to gas generation by the yeast. The doughs during long mixing become sticky and short.

When the doughs after evacuation are mixed in hydrogen or nitrogen, emulsification proceeds somewhat more slowly but approximately the same final volume of gas is emulsed at the end of 40 minutes. It is to be noted that the doughs here become soft and sticky but not short.

When oxygen is mixed in the dough there is a very surprising result; much less gas is taken up by the dough even after long mixing. However, the doughs very quickly become sticky and short. It appears that when doughs become short they lose their capacity of emulsifying gas during mixing. In the case of oxygen the dough probably becomes short so quickly that the amount of gas emulsed remains low. The vacuum-mixed dough lost a small amount of gas progressively during the mixing and gave a smooth, dense dough.

These data indicate that emulsification is progressive with mixing unless oxygen acts upon the dough. They also indicate that the stickiness and softness that often develop during mixing may be

explained as due to emulsed gas. The shortness that may develop in dough with mixing is apparently not entirely due to the emulsification itself but rather to emulsification in conjunction with some additional action of oxygen.

These experiments suggest that the progress of mixing doughs in ordinary laboratory or commercial practice might be followed by measuring the densities of the doughs at various stages of the mixing. No investigation has been made here to determine the possibility of such a test.

Next was studied the baking of breads from doughs mixed in vacuum for various lengths of time. All vacuum work was conducted at 28.0 inches of mercury. The results obtained with a commercial family flour (0.36% ash, 10.0% protein, on 15% moisture basis) and a high-protein Southwestern baker's flour (0.40% ash, 13.0% protein, on 15% moisture basis) are recorded in Table II. It is apparent that no change in the baking characteristics of the dough was produced by the long mixing in vacuum. The only observable difference was an increase in the smoothness of the dough. No development has occurred during vacuum mixing on any of the flours studied. Apparently one may conclude from these data that the only effect of prolonged mixing of dough in a vacuum is to bring about uniformity of the dough.

TABLE II

CHARACTERISTICS OF BREAD FROM DOUGHS MIXED IN VACUUM

Minutes of mixing	Untreated commercial family flour				
	Dough		Bread		
	When mixed	When panned	Texture	Age	Volume
3	Normal, dense	Normal	89	Green	2350
10	Normal, dense	Normal	89	Green	2450
20	Smooth, dense	Normal	89	Green	2420
30	Smooth, dense	Normal	90	Green	2370
40	Smooth, dense	Normal	89	Green	2410
	High-protein untreated Southwestern flour				
3	Normal, dense	Normal	94	Green	2450
5	Normal, dense	Normal	94	Green	2490
20	Smooth, dense	Normal	94	Green	2500
40	Smooth, dense	Normal	92	Green	2540

During long vacuum mixing some moisture is lost from the doughs. This may run as high as 6% in some experiments. In order to determine if this reduction in moisture content was affecting our results, breads were baked with amounts of moisture varying between the ranges found in long and short vacuum treatments, using only the short period of mixing. No more difference could be noted between

such loaves than was noted between the long and short vacuum loaves. Further confirmation of this result is to be found in the following experiments with gases, where no loss of moisture occurred.

In all the following experiments the same high-protein, untreated baker's flour mentioned in Table II was used; therefore results are comparable throughout.

The next experiment, data from which are shown in Table III, reveals the characteristics of bread baked from doughs mixed in various gases.

TABLE III

CHARACTERISTICS OF BREAD MADE FROM DOUGHS MIXED IN VARIOUS GASES

Gas	Minutes of mixing	High-protein untreated Southwestern flour				
		Dough		Bread		
		When mixed	When panned	Texture	Age	Volume
Air	5	Normal	Normal	94	Nearly mature	2730
Air	20	Very sticky [1]	Slightly dead	93	Old	2580
Air	40	Very short [1]	Short and dead	84	Very old	2210
Oxygen	5	Slightly sticky	Slightly sticky	97	Mature	2600
Oxygen	20	Sticky and short [1]	Rather dead	89	Very old	2280
Oxygen	40	Very sticky,[1] very short	Dead, very short	80	Extremely old	2190
Nitrogen	5	Normal	Normal	92	Green	2530
Nitrogen	20	Slightly soft	Normal	92	Green	2550
Nitrogen	40	Soft and sticky [1]	Normal	92	Green	2520
Hydrogen	5	Normal	Normal	94	Green	2430
Hydrogen	20	Slightly soft	Normal	92	Green	2470
Hydrogen	40	Soft and sticky [1]	Normal	91	Green	2420
Vacuum	20	Dense, normal	Normal	94	Green	2490

[1] Dough could not be handled.

Experiments with air give the characteristics familiar to many investigators (see Swanson and Working, 1926) [1] as being the effect of long mixing: the dough becomes sticky and soft, then dead and short; texture of the bread poor, volume low; and characteristics of the loaf like bread from old flour. Similar experiments with oxygen produce similar results, although to a greater degree. (See U. S. Patent No. 1,203,361.) [2]

When hydrogen and nitrogen [3] were used as the gases contacted with dough during mixing, the breads could be compared with and found identical to the vacuum breads in all respects. The doughs

[1] Swanson, C. O., and Working, E. B. Mechanical modification of dough to make it possible to bake bread with only the fermentation in the pan. Cereal Chem. 3: 65–83 (1926).

[2] Kohman, Hoffman, and Godfrey. U. S. Patent No. 1,203,361 (1916).

[3] Carbon dioxide is not reported in this series of studies as it was considered inadvisable to complicate this paper with effects of mixing where the pH of the dough was changed by the gas or ingredients used.

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Next was studied the baking of breads from doughs mixed in vacuum for various lengths of time. All vacuum work was conducted at 28.0 inches of mercury. The results obtained with a commercial family flour (0.36% ash, 10.0% protein, on 15% moisture basis) and a high-protein Southwestern baker's flour (0.40% ash, 13.0% protein, on 15% moisture basis) are recorded in Table II. It is apparent that no change in the baking characteristics of the dough was produced by the long mixing in vacuum. The only observable difference was an increase in the smoothness of the dough. No development has occurred during vacuum mixing on any of the flours studied. Apparently one may conclude from these data that the only effect of prolonged mixing of dough in a vacuum is to bring about uniformity of the dough.

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10	Normal, dense	Normal	89	Green	2450
20	Smooth, dense	Normal	89	Green	2420
30	Smooth, dense	Normal	90	Green	2370
40	Smooth, dense	Normal	89	Green	2410
	High-protein untreated Southwestern flour				
3	Normal, dense	Normal	94	Green	2450
5	Normal, dense	Normal	94	Green	2490
20	Smooth, dense	Normal	94	Green	2500
40	Smooth, dense	Normal	92	Green	2540

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such loaves than was noted between the long and short vacuum loaves. Further confirmation of this result is to be found in the following experiments with gases, where no loss of moisture occurred.

In all the following experiments the same high-protein, untreated baker's flour mentioned in Table II was used; therefore results are comparable throughout.

The next experiment, data from which are shown in Table III, reveals the characteristics of bread baked from doughs mixed in various gases.

TABLE III

CHARACTERISTICS OF BREAD MADE FROM DOUGHS MIXED IN VARIOUS GASES

Gas	Minutes of mixing	High-protein untreated Southwestern flour				
		Dough		Bread		
		When mixed	When panned	Texture	Age	Volume
Air	5	Normal	Normal	94	Nearly mature	2730
Air	20	Very sticky [1]	Slightly dead	93	Old	2580
Air	40	Very short [1]	Short and dead	84	Very old	2210
Oxygen	5	Slightly sticky	Slightly sticky	97	Mature	2600
Oxygen	20	Sticky and short [1]	Rather dead	89	Very old	2280
Oxygen	40	Very sticky,[1] very short	Dead, very short	80	Extremely old	2190
Nitrogen	5	Normal	Normal	92	Green	2530
Nitrogen	20	Slightly soft	Normal	92	Green	2550
Nitrogen	40	Soft and sticky [1]	Normal	92	Green	2520
Hydrogen	5	Normal	Normal	94	Green	2430
Hydrogen	20	Slightly soft	Normal	92	Green	2470
Hydrogen	40	Soft and sticky [1]	Normal	91	Green	2420
Vacuum	20	Dense, normal	Normal	94	Green	2490

[1] Dough could not be handled.

Experiments with air give the characteristics familiar to many investigators (see Swanson and Working, 1926) [1] as being the effect of long mixing: the dough becomes sticky and soft, then dead and short; texture of the bread poor, volume low; and characteristics of the loaf like bread from old flour. Similar experiments with oxygen produce similar results, although to a greater degree. (See U. S. Patent No. 1,203,361.) [2]

When hydrogen and nitrogen [3] were used as the gases contacted with dough during mixing, the breads could be compared with and found identical to the vacuum breads in all respects. The doughs

[1] Swanson, C. O., and Working, E. B. Mechanical modification of dough to make it possible to bake bread with only the fermentation in the pan. Cereal Chem. 3: 65–83 (1926).

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[3] Carbon dioxide is not reported in this series of studies as it was considered inadvisable to complicate this paper with effects of mixing where the pH of the dough was changed by the gas or ingredients used.

from these gases act differently. They become soft and sticky and at the 40-minute mixing period cannot be handled from the mixer. However, upon fermentation they always recover their normal dough character, no damage to the doughs is apparent, and normal bread is obtained with the characteristics of having been baked from freshly milled or green flour. It is not implied here that the gas mixing has returned the flour to its condition immediately after milling, but the authors intend to convey the fact that the flour appeared greener than an ordinary air-mixed dough would indicate. There are some indications that hydrogen produces characteristics that approach the green stage more closely than vacuum or nitrogen, suggesting that possibly the hydrogen reduced some of the oxidized ingredients of the flour which may have developed during storage and returned it toward its condition at the time of milling.

Apparently one may conclude from Table III that the prolonged mixing of doughs in the presence of inert gases such as hydrogen and nitrogen produces no injury to bread characteristics, but when oxygen is present very marked deterioration occurs.

In order to determine if the effect of mixing in vacuum or in gases had any action upon the yeast, experiments were conducted in which the mixings were done in the absence of yeast, after which the yeast was added just before the conclusion of the mixing period so that it could be thoroughly incorporated with the dough. Sufficient time was allowed in the fermentation of these doughs so that the total fermentation time was the same. Doughs prepared in this manner gave identical results with those in which the yeast was present at the beginning of the mixing, indicating that neither the long mixing, the vacuum, nor the gases had produced any action on the yeast.

In order to study further the relationship of the action of oxygen upon mixing, a series of experiments was devised whereby the action was intermittent. The data from these studies are recorded in Table IV.

The first dough was mixed in oxygen for 40 minutes, as shown in Table III. This became short and made a very poor loaf. The next dough was in contact with oxygen 40 minutes but was mixed intermittently, the first interval being a minute and a half to produce uniformity, followed by seven periods of one-half minute mixing with five minutes of resting between each. This gave a surprising result, for although the dough had been in contact with oxygen for 40 minutes and mixed at frequent intervals, it produced a perfectly normal loaf of bread of the same characteristics as a loaf mixed for five minutes in oxygen.

TABLE IV

EFFECTS OF OXYGEN UPON DOUGHS WITH CONTINUOUS AND INTERMITTENT MIXING

Treatment	Dough		Bread		
	When mixed	When panned	Texture	Age	Volume
Mixed 40 minutes in oxygen	Very soft and sticky	Dead, short	80	Extremely old	2190
In oxygen 40 minutes. Mixed 1½ minutes, then seven ½-minute periods with 5 minutes rest between. Total mix, 5 minutes in 40 minutes	Normal	Normal	97	Mature	2750
Mixed 5 minutes in oxygen	Slightly sticky	Normal	97	Mature	2600
Mixed 35 minutes in vacuum; then 5 minutes in oxygen	Rather sticky	Normal	95	Mature	2560

A further experiment was performed where the dough was mixed in vacuum for 35 minutes and then oxygen admitted for the last five minutes of mixing. This loaf again shows substantially the same normal characteristics as the above two loaves. One may again conclude from this work that the act of mixing a dough in a vacuum produces no effect other than to make the dough uniform; secondly, that the effect of oxygen upon a dough occurs only during the period while the dough is being mixed and does not carry over or have any further action. Probably during the fermentation of dough the carbon dioxide generated sweeps the oxygen out of the dough and prevents its further action.

Had the experiment stopped at this point these conclusions would apparently have been justified. However, because of the fact that oxygen produced an effect upon the dough, a study was made of the action of other oxidizing agents during mixing. It was found that the action of calcium peroxide and potassium persulphate was similar to that of oxygen. However, bromate performed in a different manner, as may be seen from the data given in Table V. Here the bromate was mixed with the dough in a vacuum for various lengths of time and at various concentrations, so that the action of the bromate was independent of the effects of oxygen gas. It is to be noted that when the dough is mixed in a vacuum in the presence of bromate, the dough appears unchanged. When fermentation proceeds, however, there develop characteristics similar to those produced when oxygen was the gas used, the doughs from short-duration mixing being improved and producing improved bread; however, after long mixing the doughs become short at the time of panning and produce bread which is very poor indeed. If the amount of bromate is reduced, however, during

TABLE V

BREAD FROM DOUGHS MIXED IN VACUUM

Minutes of mixing	% Bromate	Bromate added at start				
		Dough		Bread		
		When mixed	When panned	Texture	Age	Volume
5	0.002	Normal, dense	Normal	97	Over-mature	2780
20	0.002	Smooth, dense	Normal	94	Old	2750
40	0.002	Smooth, dense	Dead, short	85	Extremely old	2350
40	0.001	Smooth, dense	Slightly short	88	Old	2520
40	0.0004	Smooth, dense	Normal	94	Mature	2635
40	0.0001	Smooth, dense	Normal	95	Mature	2600
Vacuum-mixed 35 minutes, then bromate added and mixed 5 minutes						
40	0.002	Smooth, dense	Short	85	Extremely old	2380
40	0.001	Smooth, dense	Slightly short	89	Old	2560
40	0.0004	Smooth, dense	Normal	94	Mature	2630
40	0.0001	Smooth, dense	Normal	95	Mature	2640
40	No bromate	Smooth, dense	Normal	92	Green	2500

the longer period of mixing, satisfactory bread can be obtained. The ability to obtain satisfactory bread with a long period of mixing in the presence of bromate is possible only in the absence of oxygen.

To study further the relation of bromate to mixing, doughs were mixed in a vacuum for 35 minutes and then bromate added and mixed five minutes more, with the very surprising result that although the bromate was mixed for only five minutes in vacuum at the end of a previous 35-minute vacuum-mixing period, the bread looks as though the bromate had been present throughout the entire mixing period. This is in direct contrast to the previously mentioned action of oxygen. Smaller concentrations of bromate added at the end of the 35-minute mixing act in a manner similar to the same concentrations when present throughout the mixing. This result makes it impossible to accept the conclusions which our other work would indicate. Apparently the mixing of a dough in the vacuum has sensitized it so that it is receptive to the action of bromate and this enables the bromate to produce as much effect in a short time as though it had been present throughout the entire act of mixing. Apparently the dough becomes more reactive; the molecules have been oriented and are in a receptive condition for the bromate. The effect of mixing is stored up so that the bromate is able to produce as much effect as though it had been

present all the time. The action of bromate doubtless continues during the entire fermentation and proofing periods, up to the baking time itself. But one must conclude that the action of bromate was determined by the mixing.

A further observation may be drawn from the data in Table V. Although the dough can be sensitized by mixing so that bromate will produce very damaging effects, this same sensitized dough is capable of being markedly improved by bromate when smaller amounts are used. The maximum improvement on sensitized dough was produced by a very small amount of bromate, namely, 0.0001%. A dough mixed in vacuum in which no bromate is used is recorded alongside of this loaf. However, the amount of improvement produced in sensitized vacuum doughs by small amounts of bromate has never been as great as was produced by regular amounts of bromate used in doughs that were mixed for a short period of time, as shown by the five-minute loaf at the top of the chart, where a better texture and volume were obtained. It may possibly be that further work with smaller increments and concentrations of bromate will find a point in which further improvement is obtained on a sensitized dough; however, on all other work the authors have done of this character, which includes a number of experiments, no such result has been obtained. It appears then that the long vacuum mixing, in addition to having sensitized the dough so that bromate could completely ruin the baking quality of the dough, has subtracted something from the dough so that it cannot, by lesser amounts of bromate, be improved to the amount it would normally have been by bromate and less mixing.

It is hoped that much more work may be done on this subject in studying other variables and their relation to mixing. It is felt that the vacuum method of experimenting gives one a technique whereby one variable at a time may be investigated, whereas in the ordinary mixing of doughs in the presence of air one is always faced with the necessity of considering other variables acting upon the dough at the same time, namely, the ingredient being studied, oxygen, the emulsification of air, and the mechanical action, thereby making it very difficult to arrive at a correct conclusion.

The observations reported here upon one flour have been confirmed by repeating the salient points of the experiments upon a series of flours from various districts of the country. The results of these baking tests are shown in Table VI. In all cases the conclusions here reached have been supported and confirmed, all flours to a greater or lesser degree responding in the same manner to mixing and influence of oxidizing agents.

TABLE VI

SUMMARY OF BAKING TESTS WITH DIFFERENT FLOURS

Details of mixing	Dough		Bread		
	Mixing	Panning	Texture	Age	Volume
High-Protein Southwestern Patent Flour					
Ash 0.40%, protein 13.0%, 15% moisture basis					
3 minutes vacuum	Dense, normal	Normal	94	Green	2450
3 minutes air	Normal	Normal	96	Green	2570
3 minutes air + 0.002% bromate	Normal	Normal	97	Mature	2690
40 minutes air	Short, sticky [1]	Short, dead	84	Old	2210
40 minutes vacuum	Dense, smooth	Normal	92	Green	2540
40 minutes vacuum + 0.002% bromate	Dense, smooth	Short, dead	80	Old	2020
40 minutes hydrogen	Soft, sticky [1]	Normal	91	Green	2420
Spring Wheat Straight Flour					
Ash 0.48%, protein 11.7%, 15% moisture basis					
3 minutes vacuum	Dense, normal	Normal	92	Green	2370
3 minutes air	Normal	Normal	91	Green	2470
3 minutes air + 0.002% bromate	Normal	Normal	97	Mature	2600
40 minutes air	Short, sticky [1]	Short, dead	85	Old	2170
40 minutes vacuum	Dense, smooth	Normal	92	Green	2340
40 minutes vacuum + 0.002% bromate	Dense, smooth	Short	85	Old	2340
40 minutes hydrogen	Soft, sticky	Normal	91	Green	2310
High-Protein Texas Patent Flour					
Ash 0.40%, protein 13.6%, 15% moisture basis					
3 minutes vacuum	Dense, normal	Normal	93	Green	2460
3 minutes air	Normal	Normal	96	Mature	2650
3 minutes air + 0.002% bromate	Normal	Normal	98	Mature	2800
40 minutes air	Short, sticky [1]	Short, dead	88	Old	2310
40 minutes vacuum	Dense, smooth	Normal	94	Green	2410
40 minutes vacuum + 0.002% bromate	Dense, smooth	Short, dead	85	Very old	1970
40 minutes hydrogen	Soft, sticky [1]	Normal	91	Green	2400

TABLE VI (*Continued*)

Ohio Milled Southwestern Flour

Ash 0.46%, protein 11.0%, 15% moisture basis

3 minutes vacuum	Dense, normal	Normal	94	Green	2495
3 minutes air	Normal	Normal	95	Mature	2380
3 minutes air + 0.002% bromate	Normal	Normal	97	Mature	2480
40 minutes air	Stringy, sticky [1]	Short, dead	90	Old	2420
40 minutes vacuum	Dense, smooth	Normal	93	Green	2500
40 minutes vacuum + 0.002% bromate	Dense, smooth	Slightly dead	87	Old	2385
40 minutes hydrogen	Soft, sticky [1]	Normal	92	Green	2200

Pacific Coast Baart Wheat Patent Flour

Ash 0.42%, protein 10.9%, 15% moisture basis

Details of mixing	Dough		Bread		
	Mixing	Panning	Texture	Age	Volume
3 minutes vacuum	Normal, dense	Normal	83	Very green	2400
3 minutes air	Normal	Normal	86	Very green	2410
3 minutes air + 0.002% bromate	Normal	Normal	98	Mature	2900
40 minutes air	Short, sticky [1]	Slightly short	92	Old	2420
40 minutes vacuum	Dense, smooth	Normal	88	Green	2410
40 minutes vacuum + 0.002% bromate	Dense, smooth	Short, dead	87	Very old	2100
40 minutes hydrogen	Soft, sticky [1]	Normal	88	Green	2450

Untreated Commercial Family Flour

Ash 0.36%, protein 10.0%, 15% moisture basis

3 minutes vacuum	Dense, normal	Normal	89	Green	2410
3 minutes air	Normal	Normal	95	Slightly green	2450
3 minutes air + 0.002% bromate	Normal	Normal	96	Mature	2640
40 minutes vacuum	Dense, smooth	Normal	89	Green	2410
40 minutes vacuum + 0.002% bromate	Dense, smooth	Short	85	Extremely old	1930

[1] These doughs could not be handled.

Discussion

Many theoretical ideas may be advanced to explain the foregoing work. It is obvious that the theories which have been propounded in the past regarding the effect of mechanical action upon dough must

be modified to the extent that the action observed is the result of oxidation stimulated by the mechanical action, it apparently being necessary in order to produce modification of dough to have present both the mechanical action and the oxidizing agent at some step in the process.

The apparent difference between bromate on the one side and oxygen and inorganic peroxides on the other might be explained by the ability of bromate to remain active in the dough throughout the entire period of mixing and subsequent fermentation, whereas the other reagents—oxygen and peroxide—are removed either by aeration of the oxygen from the dough by the escaping carbon dioxide or the decomposition of the peroxide by catalase into oxygen and its subsequent removal by aeration. However, these theories require the assumption that the reactions produced by oxidizing agents are slow; otherwise a dough which has been activated by mixing should produce a similar effect with any of the oxidizing reagents in the same period. If the reactions are slow then bromate, being the only one capable of existing in the dough, is the only reagent capable of producing the effects which mixing has made possible. There may be other reagents, such as ascorbic acid, which can produce effects similar to bromates.

The fact that mixing alters the dough in such a manner that its effect can be detected by the use of bromate suggests that the mixing has arranged the molecules or particles of the dough in reactive relationship to each other, which relationship once established is retained even though the mixing continue. It is very difficult to picture what happens in the dough that makes this possible. Undoubtedly the enormous protein molecules of the colloidal mass have reactive groups which are ordinarily held in stationary position by the plastic mass so that mechanical action is necessary to roll them about and bring them into reactive relationship. It is difficult to conceive how these large molecules can combine and produce desirable effects upon the dough, for often when doughs improve during fermentation there is a softening of the dough mass. One would expect, if these large aggregates were combining, a stiffening of the mass would occur. A more acceptable theory would be a splitting of the colloidal aggregates or to conceive of a substance or conditions being produced in the dough whereby the particles passed each other more readily and the elements of friction within the dough were reduced by the action of the mixing and of the oxidizing reagent. Such a result might be produced through an effect upon some ingredient that has a lubricating action or the change may have some relationship to the starch or fat. Proposed theories which explain the changes in dough properties as due to the action of proteolytic enzymes or their activators do not appear to fit all the results

of these experiments. In the present state of knowledge of dough chemistry, speculative theories lack sufficient foundation of fact to establish any of them definitely.[4]

Summary

Doughs have been mixed in vacuum and in the presence of air, oxygen, nitrogen or hydrogen. The densities of these doughs have been determined, their handling characteristics noted, and bread has been baked from them, so that the effect of the mixing under these conditions could be studied. Doughs under vacuum have been studied in the presence of bromate and inorganic peroxides in order to aid in interpreting the results obtained when oxygen was present.

The maximum densities of doughs are obtained when mixed in vacuum, giving values of approximately one and one-quarter. When gases are present they are progressively emulsed with the dough during the mixing to such a degree that some doughs may contain 20% of their volume as emulsed gas, giving densities as low as 1.00. When oxygen is present the emulsification may not proceed as readily or as far as in its absence.

Vacuum doughs can be handled up to the longest mixing time tried and always appear dense and smooth. When hydrogen and nitrogen are present the doughs after a short mixing may handle as normal doughs but upon long mixing they tend to become sticky and soft, and the doughs cannot be handled. When oxygen is present the same characteristics are shown with the additional development of shortness. With pure oxygen these characteristics all develop more rapidly than when air is used.

The bread obtained from a vacuum dough or from dough mixed in the presence of nitrogen or hydrogen is normal bread, whether mixed for a long or short time. Apparently the mixing produces no change in the baking characteristics of the dough under these conditions, with the possible exception that hydrogen has a slight tendency to develop characteristics of bread baked from freshly milled flours. Doughs mixed in the presence of oxygen, inorganic peroxides or bromate give improvement followed by deterioration well known to the industry.

When oxygen or inorganic peroxides are added to a dough after an extended period of vacuum mixing the effects produced are the same as though the extended pre-period of mixing had not taken place. When bromate is added at the end of an extended period of vacuum mixing a

[4] Since this paper was presented before the annual convention of the A.A.C.C. there has appeared an article by Freilich and Frey, Industrial and Engineering Chemistry, News Edition 15, 294 (1937). They state: "The incorporation of oxygen into bread doughs inhibits proteolytic activity of the papain type in dough, and the oxygen does not act on the enzyme directly. The inhibition of proteolytic activity is directly proportional to the amount of flour treated with oxygen."

different action is obtained. Bromate acts as though it had been present during the entire period of mixing and produces effects entirely different from what bromate would have produced had not the pre-period of mixing occurred. Apparently the mixing has sensitized or oriented the dough so that it is capable of intensifying the bromate action during the subsequent mixing and fermentation to produce effects that would not otherwise have occurred.

Conclusions

Gases are progressively emulsed during the period of mixing dough. Oxygen may alter the progress of this emulsification.

Doughs which are mixed in vacuum or in inert gases show no deterioration from continued long mixing. Such deterioration occurs when oxygen or oxygen-yielding compounds are present.

The action of bromate in a dough is affected by the amount of mixing the dough has received, which mixing may be in the presence or absence of bromate.

EXPERIMENTS IN DETERMINING BREAD FLAVOR

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(Read at the Annual Meeting, May 1937)

Flavor is defined in Webster's New International Dictionary as "that which imparts to anything a peculiar odor or taste; gratifying to the sense of smell or the nicer perceptions of the palate." In order to be able to test a foodstuff for flavor we must consider both its taste and its smell or aroma. This is no easy thing to measure scientifically. We must rely on human beings for our tests, and the separate tests must be repeated sufficient times to insure the preference of the judges.

The American Institute of Baking has always been interested in bread flavor and factors affecting flavor in bread. In June, 1935 experiments on bread flavor were started in earnest. The purpose was to determine what constitutes good flavor (taste and aroma) in bread, and to determine, if possible, the preference of consumers apart from palatability, if that could be done.

Pelshenke (1935) has shown that the principal factors influencing flavor are time, temperature, rate, duration, and nature of fermentation; baking temperature and baking time; type of flour; and type of loaf. By testing these and other factors, it was hoped to determine whether there is any difference in the flavor of our bread today and the so-called "bread mother used to bake." However, it must be remembered, as Katz (1937) has pointed out, that impressions of bread of many years ago cannot be relied upon to any great extent.

Experience has shown that the flavor of bread varies with the consumer preference and the locality. The consumption of bread will undoubtedly increase if the combination of variables, which consumers in each locality prefer, can be found.

The procedure used in determining bread flavor and as reported in this article was similar to that described by King, Coleman, and LeClerc (1937) and Ingels, Irwin, and Landis (1936). The bread was prepared varying one factor at a time. Samples of the bread were submitted under code to the judges for testing. The results were then analyzed.

Ingels, Irwin, and Landis (1936) point out that in judging taste and aroma in bread greatest care in the preparation and age of samples is necessary. In other words, every possible effort must be made in

order to make the different samples as uniform as possible; and, at the testing stage, any factor which might cause a variation in the results must be eliminated, if possible.

Preliminary Experiments

King, Coleman, and LeClerc (1937) sliced the bread and gave the judges three samples to test at one time.

In the preliminary experiments carried out at the Institute,[1] the judges tested three different samples at one time; however, they were given only the crumb of the bread, the crust having been removed. Tests have shown that a true test of taste and aroma will not be obtained if the judges are allowed to taste the crust of the bread. This is one variable eliminated. Observations at the Institute have also shown that a good crumb will always have a good crust. Thus, the crumb is our main interest.

One may object to this way of proceeding, since bread in general is eaten either with other dishes or is used for sandwiches or with butter, etc., and is seldom eaten dry. Experiments with judges have shown that the flavor of bread will manifest itself more clearly and distinctly if it is offered plain, and it is undoubtedly correct to assume that the sample of bread, which in our experiment is best liked, will keep this superiority if it is used for sandwiches, etc. This same assumption was made recently by Katz (1937).

After about fifty separate tests were completed and the results analyzed, they were found to be somewhat conflicting and not as reproducible as they might have been. The method was altered, therefore, and thereafter the judges were given only two samples to test at one time.

This change was quite significant, for in most cases the results were found to be quite reproducible, and the order in which the bread was tested did not make any difference. When three samples were tested at one time, the order of testing the samples significantly affected some of the results. This same effect was noted by King, Coleman, and LeClerc (1937). This and other experiments indicate that a person can make his best and most accurate differentiation when judging only two samples.

Since the consumer does not differentiate to any great extent between taste and aroma, it was thought best to have the judges combine the two and report them as one. Although they would actually examine each sample for aroma and then for taste, they combined their opinion of these two factors into the flavor of the sample.

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[1] Experiments by W. Walmsley, R. A. Nelson, and P. R. Beier.

Judges

The judges included the members of the staff of the Institute, who have had experience in judging bread flavor; the students of the Institute, who have received training in judging bread flavor; and the clerical staff, members of which have had very little training in the subject. The students at the Institute represent 22 States of the United States. No attempt was made to make everyone in the building take the tests; thus, only those who were interested in what was trying to be done were the judges. An average of about 25 votes was received in each test.

Ingels, Irwin, and Landis (1936), Maiden (1936), and Moir (1936) have recommended that some method be employed for selecting judges with discriminating palates for flavor tests. Although no attempt was made to choose only judges whose senses of smell and taste were keen, a group of seven of the judges were tested and selected for their keen senses of smell and taste. This was accomplished by having them examine different concentrations of chemically pure solutions of sugar, salt, acetic acid, and caffeine. It was found that the results of this group were practically the same as the results of all the judges.

Selecting judges for their keen sense of smell and taste may be perfectly justified for flavor tests of some kinds of foodstuffs and when small differences in flavor are sought; however, for the purpose of this research it does not seem justified. First, a selection of judges seemed to make no difference in the results; second, bread flavor is a neutral flavor and is a blend of the primary tastes (sweet, bitter, acid, and saline) and a great variety of smells, and not a matter of selecting one taste or smell; and third, the consumers who buy the bread are not selected judges.

In answer to a question asked by the author at the 1937 Annual Meeting of the American Society of Bakery Engineers, W. E. Broeg is of this same opinion.

The degree to which the judges were able to reiterate their findings when successive tests were made on the same experiments should confirm their ability to judge bread flavor.

Maiden (1936) raises the question whether it is justifiable to consider aroma and taste as one and the same thing. They are not identical, and that is just why the judges were instructed to test the bread samples for both aroma and taste.

One error that can not be controlled is the variation in individual taste from day to day. This is a factor of considerable importance but must be accepted as such.

Procedure

Unless otherwise stated, the doughs were machine mixed at medium speed, fermented at 78° to 82° F., made up by hand into round-top loaves, proofed at 90° to 95° F., scaled at 18 oz., and baked in single pound-size pans at 450° F. The relative humidity of the fermentation room was 70% to 75%, and that of the proof box 80% to 85%. After leaving the oven the loaves were allowed to cool until the inside crumb was at least 90° F., and then nested together and covered with a white porous cloth. Unless otherwise stated, they were stored at room temperature in this manner until time for sampling. Most of the samples were tested when 24 hours old.

The bread was sliced by hand, the crust cut off, and immediately placed in sterile, covered glass jars, labeled No. 1 and No. 2. The two jars containing the two different samples were then placed on a table near a water fountain, at a convenient point in the building for testing. Ballots and a ballot box were placed on the same table. A large sign carried the following notice:

NOTICE

1. Please test the bread for AROMA and TASTE alone.
2. Disregard all other factors.
3. Between each test, take a DRINK OF WATER.
4. Mark ballot with number corresponding with that on the jar which contains your choice.

Only two samples were tested at one time. All of the judges followed the rules strictly and simply chose the sample they thought had the best flavor (combined taste and aroma). The judges had the choice of using as many pieces of bread as they desired for the test, and they could also test either sample first. When the tests were repeated, the samples were always reversed, *i.e.*, No. 1 was changed to No. 2, and *vice versa*. Although, as mentioned before, it did not matter which sample was tested first, this procedure of reversing the samples would help to eliminate this error if such should exist.

When tests were repeated, several days and sometimes months were allowed to elapse between tests. This procedure tends to make the tests more reliable.

Every effort was made to insure only one variable in each test.

Experimental

For purposes of these tests, the formulae are indicated in Table I as No. 1, No. 2, No. 3, and No. 4. These formulae increase in richness

TABLE I

SCHEDULE OF EXPERIMENTS AND TESTS AND RESULTS

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
1. *Types of Flour:*									
(a) Long extraction soft wheat	30	40				Straight dough	1	7	24
(a) 85% extraction hard wheat (high grade patent from high protein wheat)	70	60							
(b) Blend of 1/3 long extraction soft wheat and 2/3 85% extraction hard wheat	45	44	52			Straight dough	1	Test 1, 2—7 Test 3—20	24
(b) 85% extraction hard wheat	55	56	48						
(c) Blend 1/2 long extraction soft wheat and 1/2 85% extraction hard wheat	50	37	29			Test 1—50/50 Sponge dough (soft wheat in dough) Test 2, 3—Straight dough	1	7	24
(c) 85% extraction hard wheat	50	63	71						
(d) Blend 2/3 long extraction soft wheat and 1/3 85% extraction hard wheat	50	57	12	47	00	Straight dough	1	Test 1, 2, 3—7 Test 4, 5—20	24
(d) 85% extraction hard wheat	50	43	88	53	100				
(e) Blend 1/2 high grade cake flour and 1/2 85% extraction hard wheat	41	58				Straight dough	1	20	24
(e) 85% extraction hard wheat	59	42							

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
(f) Blend 1/3 high grade cake flour and 2/3 85% extraction hard wheat	33	52				Test 1—60/40 Sponge dough Test 2—Straight dough	1	Test 1—7 Test 2—20	24
(f) Blend 1/3 long extraction soft wheat and 2/3 85% extraction hard wheat	67	48							
(g) Blend 1/2 high grade cake flour and 1/2 85% extraction hard wheat	33	40				Straight dough	1	20	24
(g) Blend 1/2 long extraction soft wheat and 1/2 85% extraction hard wheat	67	60							
2. *Bleaching of Flour:*									
(a) Bleached (85% patent as before)	50	42*	31*	29*	39*	Straight dough	1	7	24
(a) Unbleached (85% patent as before)	50	58	69	71	61				
(b) Bleached (85% patent as before)	23	43*	59			Test 1—60/40 Sponge dough Test 2, 3—Straight dough	1	7	24
(b) Unbleached (long extraction practically a straight)	77	57	41						
3. *Absorption:*									
Stiff 56 % Water	39*	40*	72	59		Straight dough	1	7	Test 1, 2, 3—24
Normal 62 Tests 1, 2, 3, and 4	31	40							Test 4—72 wrapped in wax
Slack 68	30	20	28	41					

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
4. *Type of Formula:*									
(a) Formula 1	52	50				Test 1—16-hour 60/40 Sponge dough Test 2—Straight dough	—	Test 1—7 Test 2—20	3
Formula 3	48	50							
(b) Formula 1	63	75	60	67		Test 1—16-hour 60/40 Sponge dough Test 2, 3, 4—Straight dough	—	Test 1, 2, 3—7 Test 4—20	24
Formula 3	37	25	40	33					
(c) Formula 1	46	65				Straight dough	—	Test 1—7 Test 2—20	48
Formula 3	54	35							
(d) Formula 1	39					60/40 Sponge dough	—	30	72 wrapped in wax
Formula 3	61								
(e) Formula 1	52					60/40 Sponge dough	—	30	3
Formula 4	48								
(f) Formula 1	76					60/40 Sponge dough	—	30	24
Formula 4	24								
5. *Method of Mixing:*									
(a) Hand mixed (greatly undermixed)	33					Straight dough	1	7	24
Machine mixed (normal)	67								

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
(b) Hand mixed (very well mixed) Machine mixed (normal)	69 31	58 42				Straight dough	Test 1—1 Test 2—2	Test 1—7 Test 2—15	24
(c) Machine mixed (slightly undermixed) Machine mixed (slightly overmixed)	60 40					Straight dough	1	7	24
(d) Machine mixed (normal mixing) Machine mixed (greatly overmixed)	70 30					Straight dough	1	7	24
(e) Machine mixed (greatly undermixed) Machine mixed (normal mixing)	35 65	41 59				Straight dough	2	20	24
(f) Machine mixed (low speed 36 R.P.M.) Machine mixed (high speed 72 R.P.M.)	48 52	56 44	59 41			Straight dough	2	100	24
6. *Make-up:* (a) Machine mixed (hand make-up) Machine mixed (normal machine make-up)	64 36	64 36	78 22	64 36		Straight dough	2	100	24
(b) Machine mixed (semi-brake dough) Machine mixed (standard dough)	43 57					Straight dough	2	75	24
(c) Machine mixed (brake dough) Machine mixed (standard dough)	15 85	27 73				Test 1—60/40 Sponge dough Test 2—Straight dough	2	100	Test 1—24 Test 2—48

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
7. *Punching of Straight Dough:*									
No punches	54	54				Straight dough	2	100	24
Normal number of punches	46	46							
8. *Methods of Fermentation:*									
(a) 60/40 sponge dough	41	45				—	2	30	24
80/20 sponge dough	59	55							
(b) 60/40 sponge dough	29	35				—	2	30	24
Straight dough	71	65							
(c) 60/40—13-hour sponge dough	37	31				—	2	30	24
Straight dough	63	69							
(d) 50/50 process dough	50	39				—	2	100	Test 1—3 Test 2—24
Straight dough	50	61							
(e) 80/20 process dough	48	45	48			—	2	Test 1, 2—100 Test 3—75	24
Straight dough	52	55	52						
(f) 60/40 80° F. sponge and 80° F. dough	52	67				—	2	75	24
60/40 88° F. sponge and 80° F. dough	48	33							
(g) Straight dough (80° F.)	69					—	1	40	24
60/40 89° F. sponge and 80° F. dough	31								
(h) 60/40 80° F. sponge and 80° F. dough	69	62				—	2	30	24
60/40 88° F. sponge and 88° F. dough	31	38							

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
(i) 60/40 80° F. sponge and 80° F. dough 60/40 80° F. sponge and 90° F. dough	63 37	60 40				—	2	20	24
(j) 70/30 foam sponge Straight dough	48 52	83 17	74 26			—	Test 1, 2—2 Test 3—1	50	24
(k) 15-minute straight dough 82° F., 5% yeast Straight dough	38 62	32 68				—	2	50	24
(l) 15-minute straight dough 90° F., 2.5% yeast Straight dough	22 78	35 65				—	2	50	24
(m) Progressive sponge Straight dough	32 68	26 74				—	1	60	24
9. *Proof Time:* (a) 25 minutes underproof Normal proof time	42* 58	40 60				Straight dough	2	20	24
(b) 25 minutes overproof Normal proof time	41* 59	38 62				Straight dough	2	20	24
10. *Size of Baking Pans:* (a) 4.43 cubic inches per ounce of dough 5.68 cubic inches per ounce of dough	45 55					60/40 Sponge dough	2	100	24

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
(b) 4.43 cubic inches per ounce of dough 5.97 cubic inches per ounce of dough	% 53 48	% 43 57	% 42 58	%	%	Test 1—Straight dough Test 2, 3—60/40 Sponge dough	Test 1—1 Test 2, 3—2	Test 1—30 Test 2, 3—100	24
(c) 4.43 cubic inches per ounce of dough 6.37 cubic inches per ounce of dough	33 67					60/40 Sponge dough	2	100	24
(d) 4.43 cubic inches per ounce of dough 7.19 cubic inches per ounce of dough	36 64					60/40 Sponge dough	2	100	24
(e) 5.97 cubic inches per ounce of dough 6.37 cubic inches per ounce of dough	56 44					Straight dough	2	15	24
(f) 5.97 cubic inches per ounce of dough 7.19 cubic inches per ounce of dough	56 44	54 46				Straight dough	Test 1—1 Test 2—2	20	Test 1—24 Test 2—12
11. *Baking Conditions:* (a) Round top Twist	67 33	73 27	77 23			Test 1, 2—60/40 Sponge dough Test 3—50/50 Process dough	2	Test 1—20 Test 2, 3—30	24
(b) Round top Cross pan	55 45	52 48	41 59			50/50 Process dough	2	Test 1, 2—30 Test 3—100	24
(c) Round top Pullman	55 45	45 55	53 47	55 45		Straight dough	2	20	24
(d) Baked 25 minutes at 500° F. Baked 55 minutes at 400° F.	36 64					60/40 Sponge dough	2	100	24

TABLE I—*Continued*

Experiment	Test number					Dough process	Formula number	Pounds of flour in batch	Hours between baking and testing
	1	2	3	4	5				
	%	%	%	%	%				
(e) Baked 35 minutes at 480° F. Baked 65 minutes at 400° F.	40 60					60/40 Sponge dough	2	100	24
(f) Round top Hearth baked	67 33	68 32	79 21			Test 1, 2—60/40 Sponge dough Test 3—Straight dough	Test 1, 2—2 Test 3—1	Test 1, 2—100 Test 3—20	24
12. *Freshness of Baked Product:* (a) 4 hours old 12 hours old	40 60	38 62				Straight dough	2	20	—
(b) 4 hours old 24 hours old	43 57					60/40 Sponge dough	2	100	—
(c) 4 hours old 48 hours old	62 38					60/40 Sponge dough	2	100	—
(d) 4 hours old 72 hours old (wrapped in wax)	56 44					60/40 Sponge dough	2	100	—
(e) 12 hours old 24 hours old	81 19					Straight dough	2	20	—
(f) 12 hours old 48 hours old	68 32	60 40				Straight dough	2	20	—
(g) 12 hours old (wrapped in wax) 72 hours old (wrapped in wax)	63 37					60/40 Sponge dough	2	100	—

* Experiments by Walmsley, Nelson, and Beier.

according to number as follows:

	Formula number			
	1	2	3	4
	%	%	%	%
Flour	100	100	100	100
Absorption	—	—	—	—
Sugar	2	4	6	10
Salt	2	2	2	2
Shortening	2	3	6	10
Malt	1	1	1	1.5
Milk powder	2	5	6	5
Yeast	2.5	2.5	2.5	3
Yeast food	0.25	0.25	0.25	0.25

In speaking of the dough processes the ratio used in connection with these indicates the amount of flour in the sponge (numerator) and the amount of flour in the dough (denominator). For example, a 60/40 sponge dough means that 60% of the flour was put in at the sponge stage and 40% at the dough stage.

Unless otherwise stated a straight dough refers to a dough that is fermented for approximately 3½ hours and a sponge dough refers to a dough that is fermented for approximately 4 hours.

No tests were made on different grades of flour. A high grade bleached patent (approximately 85% extraction from a high protein wheat) was used throughout the experiments. King, Coleman, and LeClerc (1937) found this extraction to be preferable to a 70% extraction or to a straight. In the experiments on types of flour and bleaching of flour the above mentioned patent flour was used as a standard for comparison.

A schedule of the experiments shown as Table I gives the type of dough process, type of formula, pounds of flour in the formula, and age of samples when tested.

Only the best of materials were used throughout the tests and the loaves were always of good quality.

1. *Types of flour.*—The analysis of the flour used is as follows:

	Absorption	Moisture	Ash	Protein
	%	%	%	%
Long extraction soft wheat (local)				
As received	58	12.4	0.400	9.35
15% moisture basis	53.3	—	0.388	9.07
85% extraction hard wheat (blend) milled from high protein wheat (average of 3 samples)				
As received	64	12.3	0.446	13.00
15% moixture basis	58.9	—	0.433	12.60

Although the work of Smith (1928) indicates that better bread will result from soft wheat flour when a rich formula is used, all of these tests with soft wheat flour were made using a lean formula.

In experiment 1 (see Table I), the fermentation times for all the hard wheat flour samples were normal. The soft wheat flour samples required a shorter time depending on the amount of soft wheat flour in the blend. In the case of 100% soft wheat flour this was much shorter. In all the tests the soft wheat samples (made from the long extraction sample) were good loaves and compared with the hard wheat samples in every way, except for a lighter bloom.

The fact that test 1 in experiment 1 (c) does not check test 2 and test 3 can not be blamed entirely on the judges, for the sponge and dough method was employed in this test while the bread in tests 2 and 3 was made by the straight dough method. The sponge is given sufficient time to mature and develop a great deal of flavor; the dough stage (at which point the soft wheat flour is added) is given very little time. This may also be the reason for the tests in experiment 1 (f) and 2 (b) not checking. Undoubtedly the dough process employed makes more difference when soft wheat flour is concerned than otherwise.

2. *Bleaching of flour.*—The long extraction unbleached flour used in the experiment was an exceptionally high protein flour. The analysis is as follows:

	Absorption	Moisture	Ash	Protein
	%	%	%	%
As received	64	11.6	0.495	15.62
15% moisture basis	57.3	—	0.476	15.02

3. *Absorption.*—The flour used was the regular high grade 85% patent.

4. *Type of formula.*—The flour was the same as in the previous experiment.

In experiments 4 (a) and 4 (e) it has been noted that there was no significant difference. In this and other experiments, it was found that bread three to four hours out of the oven showed smaller differences than bread 24 hours old; *i.e.*, if the bread was nested together and covered, but not wrapped in the conventional manner. The gassy or fermentation aroma of three- or four-hour old bread seems to cover up all other flavors.

5. *Methods of mixing.*—The flour used was the same as in the previous experiment.

The tests in experiment 5 (f) were made on a commercial scale and were handled in like manner. They were all mixed in a small-size horizontal commercial mixer with two speeds (36 r.p.m. and 72 r.p.m.).

In case of all experiments in this paper, a normal mixing period was that which had been found to give best results with the flour used. This corresponded with the point of clean-up in the mixer.

6. *Make-up.*—The flour used was again the same.

The tests on make-up were again made on a commercial scale in the completely equipped bakery at the American Institute of Baking.

7. *Punching of straight dough.*—The flour used was the same as before.

8. *Methods of fermentation.*—First, let us describe briefly some of the various dough processes used which might not be familiar to everyone.

In the process dough (sometimes called the soaker method) all ingredients except the yeast are put in the sponge and left for 3 hours and 15 minutes. This sponge is then put back into the mixer with the yeast and then treated as a normal straight dough. The amount of flour in the sponge can be varied as in any sponge.

In the foam sponge (sometimes called the flying sponge) all water, malt, milk, yeast, and yeast food are put in the sponge. This is left 15 minutes, mixed into the dough with the remainder of the ingredients and immediately taken to the bench.

The progressive sponge and dough method was put in because it is supposed to give a loaf with excellent flavor, especially when clear flour, potato flour, and buttermilk are used, as in the following formula:

PROGRESSIVE SPONGE DOUGH

85° F. Overnight sponge			85° F. Sponge			Dough		
	Lb.	*Oz.*		*Lb.*	*Oz.*		*Lb.*	*Oz.*
1st clear	9		1st clear	9		Patent flour	33	
Water	6	12	Patent flour	9		Water	20	
Yeast		3	Water	11	4	Salt	1	2
Malt		5	Malt		2.5	Malt		2.5
Yeast food		2.5				Shortening		9.75
Potato flour	1	13				Yeast		8
						Dry buttermilk		9.75

The flour used was the same as before and almost all tests were made on a commercial scale.

In making hot sponge and dough products, it must be pointed out that the various operations must be performed on time.

9. *Proof time.*—The flour was the same as before.

10. *Size of pans.*—The same flour as before was used and most experiments were on a commercial scale.

In all tests of experiment 10 the doughs were scaled at 18 ounces and different pan sizes used.

The pan sizes are given in cubic inches per ounce of dough. It takes a difference of about 2 cubic inches per ounce of dough to make enough difference in the flavor for the judges to detect it.

11. *Baking conditions.*—The flour was the same as in the previous tests.

All tests except one in experiments 11 (d), 11 (e), and 11 (f) were made on a commercial scale.

12. *Freshness of baked product.*—The flour was the same as before.

Summary

This paper tabulates the impartial reaction of one group of people who acted as judges as to bread flavor (aroma and taste) in approximately 124 separate tests—a relatively small number when the universal use and appeal of the product are considered. The method of testing is given. A number of factors were covered. The tests point out that other factors besides ingredients influence bread flavor.

One purpose of this study has been to work out a practical method of tests that may be used by any baker or miller in any part of the country to determine taste preferences of consumers in his own territory. The results of the tests in one territory may not be useful to a baker or miller in another, as it is recognized that tastes are different in different territories. But the plan or method [2] of making these tests and obtaining useful results can be applied in any territory.

It must be emphasized that the tests tabulated here are the result of a series of experiments at one point with one group of judges and with limited variation in ingredients and processes. Results may vary with different sets of judges in different parts of the country. Conclusions have not been drawn for these reasons.

Acknowledgment

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STUDIES ON THE BAKING QUALITY OF FLOUR AS AFFECTED BY CERTAIN ENZYME ACTIONS. IV. POTASSIUM BROMATE AND ENZYME ACTIVITY

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Introduction

That normal bread flour contains at least two protease factors is generally accepted. Unfortunately, our present chemical methods for quantitatively determining the extent of proteolysis which may occur during baking processes are inadequate. In three preceding papers (Read and Haas, 1934, 1936, 1937) the authors have presented data which show the benefits produced by small increments of certain proteases on the baking quality of bread flours giving rise to "bucky" doughs.

The problem of activating and inactivating enzymes, as related to the baking industry, has recently received attention. The information to be gained by such studies should be of practical value. The use of minute quantities of potassium bromate as a "dough improver" has been practiced for about two decades. The favorable response often obtained by its use has stimulated investigation concerning the actual nature of its behavior in the dough. The experimental studies to be presented in this paper will deal chiefly with the effects of bromate (and other salts) on proteolytic activity as measured separately on flour and gelatin, also as influenced by dialysis, and by adsorption.

Review of Literature

On the basis of studies concerned with the digestion of casein (*in vitro*) by trypsin and by pancreatin, Falk and Winslow (1918) pointed out that potassium bromate in the quantity ordinarily employed for baking purposes (*i.e.*, one part of bromate to 100,000 parts of flour) very possibly exerted a specific stimulative action upon the proteolytic enzymes active in fermentation. They found that potassium bromate in concentrations varying from one part in 100,000 to one part in 200,000 consistently stimulated the digestion of casein by trypsin or by pan-

creatin, but proteolytic activity was slightly inhibited when the bromate was present to the extent of one part in 10,000.

Elion and Elion (1932), British Patent 375,679, claim that persulfate of potassium or of ammonium, in very small amounts, increases the saccharogenic activity of flour very appreciably. When applied in

TABLE I

EFFECTS OF VARYING AMOUNTS OF POTASSIUM BROMATE ON WATER SOLUBLE NITROGEN (Jørgensen Method)

Enzymic agent digested with 25 g. flour No. 150353	Amount added	Milligrams of $KBrO_3$ digested with flour sample					
		None	0.25	0.5	1	50	100
	Mg.	Milligrams N in 20 c.c. of extract					
Flour control	None	14.3	14.3	14.3	14.3	13.6	13.5
Same flour heated 12 hours at 95° C.	None	14.3	14.3	14.3	14.3	13.5	13.3
Barley malt flour No. 5	50	14.3	14.3	14.3	14.1	13.7	13.6
Wheat malt flour K	50	14.3	14.3	14.3	14.0	13.7	13.6
Cysteine hydrochloride	10	16.8	16.8	16.8	16.8	16.4	16.2
Merck's diastase of malt (U. S. P.-IX)	50	16.4	16.4	16.4	16.3	15.7	15.7
Merck's diastase of malt (U. S. P.-IX)	100	19.4	19.4	19.4	19.3	18.6	18.6
Taka diastase	50	20.3	20.3	20.3	20.2	19.9	19.7
Trypsin	50	48.0	—	48.2	—	48.0	47.0
Pepsin	50	20.1	20.1	20.0	20.1	19.2	18.8
Papain [1]	50	86.5	86.2	85.1	81.0	29.1	23.4
Papain [1]	10	43.0	43.0	42.1	—	16.7	15.3
Raw pineapple juice [1]	5 drops	46.3	46.2	43.6	43.2	16.9	—

[1] Papain and bromelin are the only proteases which appear to be inhibited by bromate when it is present in excessive amounts.

TABLE II

EFFECTS OF CERTAIN CHEMICAL AGENTS ON WATER SOLUBLE NITROGEN (Jørgensen Method)

Enzymic agent digested with 25 g. flour No. 150353	Amount added	Milligrams of chemical agent added to the digest			
		Controls	5 $NaHSO_3$	10 $NaHSO_3$	100 Vitamin C[1]
	Mg.	Milligrams N in 20 c.c. of flour extract			
Flour alone	None	14.1	16.2	16.8	14.2
Protease killed flour	None	12.9	—	15.1	
Merck's diastase of malt	80	19.0	21.2	23.8	23.0
Raw pineapple juice	12 drops	66.4	70.2	73.4	67.6
Papain	12	51.0	90.0	92.0	64.7
Taka diastase	50	24.2	—	—	27.2
Pepsin	50	19.7	—	—	24.1
Trypsin	50	47.2	—	—	45.2

[1] Merck's crystalline vitamin C (Cebione). With the exception of trypsin, Vitamin C consistently produced an increase in water soluble nitrogen over and above the controls.

amounts varying from 0.25 to 0.02% by weight of flour, the action of diastase is materially promoted.

Working (1928) explained the action of oxidizing agents on flour as a liberation of phosphatides, which in turn reacted favorably upon the gluten. Geddes (1930) suggested that the benefits derived from

TABLE III

INFLUENCE OF CERTAIN CHEMICAL AGENTS ON PROTEOLYTIC ACTIVITY; TESTS WITH AN 8% EXTRACT OF MERCK'S DIASTASE OF MALT (U. S. P.-IX)
(Digestion Period 24 Hours at 27° C.)

Amount of extract added	Temperature	Controls enzymic	Milligrams of chemical agent added to each flask					
			10 $KBrO_3$	20 $KBrO_3$	10 $NaVO_3$[1]	20 $NaVO_3$[1]	5 $NaHSO_3$	10 $NaHSO_3$
Drops	° C.	Consistency of gelatin after 24 hours' digestion at 27° C.						
1	25	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
	0	Hard	Hard	Hard	Hard	Hard	Hard	Hard
2	25	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Very firm	Very firm	Very firm	Very firm	Soft	Hard	Hard
3	25	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Firm	Medium firm	Soft	Mobile	V. visc. liquid	Very firm	Firm
4	25	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Mobile	V. visc. liquid	Viscous liquid	Viscous liquid	Liquid	Firm	Mobile
5	25	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0	V. visc. liquid	Viscous liquid	Viscous liquid	Viscous liquid	Liquid	Medium firm	Mobile
6	25	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Viscous liquid	Liquid	Liquid	Liquid	V. visc. liquid	Viscous liquid
8	25	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Liquid	Liquid	Liquid	Liquid	V. visc. liquid	Viscous liquid

[1] Treating flour with minute amounts of vanadium compounds. See British Patent 452, 483 (1936).

the use of bromate may arise from the oxidation of certain germ components believed to be detrimental, presumably the phosphatides. Later Geddes and Larmour (1933) directed attention to a possible "ionic" effect on the gluten proteins whereby the gas-retaining capacity of the gluten was increased.

Kosmin (1934) has reported interesting studies concerned with the colloidal properties of glutens as affected by such oxidizing agents as $KMnO_4$, H_2O_2, $K_2S_2O_8$, $KBrO_3$ and KIO_3. The first three compounds produced a marked damaging effect on the gluten when used in neutral 0.1 N solution, but the bromate and iodate, in equivalent amounts, produced no observable effect on the gluten until acidified. When dissolved in 0.1 N acid, the action of bromate and iodate was similar to that of the permanganate or persulfate without any acidification. All tests were

TABLE IV

INFLUENCE OF CERTAIN CHEMICAL AGENTS ON PROTEOLYTIC ACTIVITY; TESTS WITH AN 8% EXTRACT OF MERCK'S DIASTASE OF MALT (U. S. P.-IX)

Amount of extract added	Temperature	Controls enzymic	Milligrams of chemical agent added to each flask		
			10 Vitamin C	5 KSCN	30 $CaCl_2$
Drops	° C.	Consistency of gelatin after 24 hours' digestion at 27° C.			
1	25	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
	0	Hard	Hard	Hard	Hard
2	25	Liquid	Liquid	Liquid	Liquid
	0	Very firm	Very firm	Very firm	Very firm
3	25	Liquid	Liquid	Liquid	Liquid
	0	Firm	Firm	Firm	Firm
4	25	Liquid	Liquid	Liquid	Liquid
	0	Mobile medium	Mobile medium	Mobile medium	Mobile soft
5	25	Liquid	Liquid	Liquid	Liquid
	0	V. visc. liquid	Mobile soft	V. visc. liquid	Viscous liquid
6	25	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	V. visc. liquid	Viscous liquid	Liquid
7	25	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Viscous liquid	Liquid	Thinner liquid
8	25	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Liquid	Liquid	Thinner liquid

made with fat-extracted flour, thereby removing lipoids from consideration.

On the basis of her experimental results, Kosmin concluded that gluten quality as governed by "improving agents" is a function of the degree of coagulation effected by these agents. Suitable coagulation may be produced by a number of chemical substances, even by a reducing agent such as HCHO, but the total beneficial effect on quality is essentially the same regardless of the agent used to induce the desired degree of coagulation.

Potel (1934, 1935) published studies dealing with the effects produced by small quantities of various chemicals on the plastic properties of flour pastes as determined by means of the Chopin extensimeter, particularly those substances known as "improvers," such as bromate,

TABLE V

INFLUENCE OF CERTAIN CHEMICALS ON THE PROTEOLYTIC ACTIVITY OF RAW PINEAPPLE JUICE

Amount of juice added	Temperature	Controls enzymic	Milligrams of chemical agent added to each flask			
			5 $NaHSO_3$	5 KSCN	5 $KClO_3$	30 $CaCl_2$
Drops	° C.	Consistency of gelatin after 24 hours' digestion at 27° C.				
1	25	Viscous liquid	V. Visc. liquid	Viscous liquid	Viscous liquid	Viscous liquid
	0	Hard	Hard	Hard	Hard	Hard
2	25	Liquid	Viscous liquid	Liquid	Liquid	Liquid
	0	Fairly hard	Hard	Hard	Fairly hard	Fairly hard
3	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Fairly soft	Hard	Medium hard	Fairly soft	Very soft
4	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Very soft	Hard	Fairly soft	Mobile stiff	Mobile stiff
5	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Mobile	Medium hard	Mobile stiff	V. visc. liquid	V. visc. liquid
6	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	V. visc. liquid	Fairly soft	Mobile soft	Viscous liquid	Viscous liquid
7	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Very soft	V. visc. liquid	Liquid	Liquid
8	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Mobile soft	Viscous liquid	Liquid	Liquid
10	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	V. visc. liquid	Liquid	Liquid	Liquid

iodate, persulfate, perborate, and other oxidants such as Na_2O_2, H_2O_2 and $KMnO_4$ He found that bromate and iodate exerted an unfavorable action on the plastic properties, while perborate and persulfate exerted a favorable action as did also H_2O_2 and Na_2O_2.

Reducing substances and wheat germ were also investigated. Gluten washed with very dilute solutions of perborate and persulfate had greater tenacity and cohesiveness than did the gluten washed with solutions containg similar quantities of Na_2SO_3 and KCN. These latter produced slackness, filamentation and lack of cohesion. Potel reached the con-

TABLE VI

INFLUENCE OF CERTAIN CHEMICAL AGENTS ON PROTEOLYTIC ACTIVITY; TESTS WITH AN 8% EXTRACT OF PAPAIN

Amount of extract added	Temperature	Controls enzymic	Milligrams of chemical agent added to each flask			
			5 $NaHSO_3$	20 $Na_2S_2O_3$	5 $KBrO_3$	5 $NaVO_3$
Drops	° C.	Consistency of gelatin after 24 hours' digestion at 27° C.				
1	25	Viscous liquid	V. visc. liquid	V. visc. liquid	V. visc. liquid	V. visc. liquid
	0	Medium	Hard	Hard	Hard	Hard
2	25	Liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
	0	Soft	Hard	Hard	Hard	Hard
3	25	Liquid	Viscous liquid	Viscous liquid	Liquid	Liquid
	0	Mobile	Medium	Hard	Hard	Hard
4	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Soft	Fairly soft	Fairly hard	Fairly hard
5	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	V. visc. liquid	Mobile	Fairly soft	Fairly soft
6	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Viscous liquid	V. visc. liquid	Mobile stiff	Mobile stiff
7	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Liquid	Viscous liquid	Mobile soft	Mobile soft
8	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Liquid	Viscous liquid	V. visc. liquid	V. visc. liquid

clusion that there exists in wheat flour a substance associated with the gluten complex which is susceptible to oxidation and reduction.

Sullivan, Near and Foley (1936) pointed out that it is the water soluble constituents of wheat germ and not the ether soluble which injure the gluten. In a subsequent paper Sullivan, Howe and Schmalz (1936) concluded that it was the reduced glutathione in the germ and not the proteolytic enzyme which injured the gluten of their doughs.

In their opinion, the minimizing effect of oxidizing agents is produced by converting some of the S–H glutathione (reducing) to the inactive S–S form. They caution against too narrow a view regarding the activation of proteolytic enzymes by substances containing the sulf-hydryl group, because many compounds, inorganic as well as organic, can materially influence the colloidal behavior of the gluten proteins by modifying the oxidation-reduction system. Attention was called to the fact that cysteine permits of the production of a perfect loaf of bread while glutathione does not.

TABLE VII

INFLUENCE OF CERTAIN CHEMICAL AGENTS ON PROTEOLYTIC ACTIVITY; TESTS WITH AN 8% SOLUTION OF TAKA DIASTASE

Amount of solution added	Temperature	Controls enzymic	Milligrams of chemical agent added to each flask			
			5 $NaVO_3$	5 KSCN	5 $NaHSO_3$	5 $KBrO_3$
Drops	° C.	Consistency of gelatin after 24 hours' digestion at 27° C.				
1	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Soft	Soft	Soft	Soft	Soft
2	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	V. visc. liquid	V. visc. liquid	V. visc. liquid	V. visc. liquid	V. visc. liquid
3	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Viscous liquid	Viscous liquid	V. visc. liquid	Viscous liquid
4	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Viscous liquid	Viscous liquid	V. visc. liquid	Viscous liquid
5	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Liquid	Liquid	Viscous liquid	Liquid
6	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Liquid	Liquid	Viscous liquid	Liquid

Bull (1937) pointed out that the constituents of wheat germ which injure baking quality are water soluble and dialyzable, such for example as asparagine.

In a series of three papers, Jørgensen (1935, 1936) discredited the phosphatide and "electrolyte" theories and presented considerable data to support his contention that the benefits derived from bromate and other agents which behave in like manner in bread doughs are the direct result of protease inhibition. Jørgensen's studies were made with buffered gelatin solutions and buffered flour extracts. The flour extracts

were prepared as follows: 25 g. samples of flour were digested with 75 c.c. of distilled water plus 25 c.c. of phosphate buffer (pH — 5.8) for 2 hours at 35° C. The digestions were continuously shaken during the two-hour period, following which they were centrifuged, filtered, and the nitrogen (Kjeldahl) determined in 20 c.c. portions of each extract. To measure the action of bromate and other similar agents in the pres-

TABLE VIII

PROTEOLYTIC ACTIVITY AS AFFECTED BY BROMATE ACTION AND SUBSEQUENT DIALYSIS; TESTS WITH A 6% EXTRACT OF MERCK'S DIASTASE OF MALT (U. S. P.-IX)

Varying quantities (mg.) of bromate were digested with 100 c.c. of extract for 2 hours at 35° C., and then dialyzed free from bromate prior to the tests on gelatin.

Amount of extract added	Temperature	Controls not dialyzed	Dialyzed series			
			Controls dialyzed	25 $KBrO_3$	50 $KBrO_3$	100 $KBrO_3$
Drops	° C.	Consistency of gelation after 24 hours' digestion at 27° C.				
2	25	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
	0	Hard	Hard	Hard	Hard	Hard
3	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Fairly hard	Fairly hard	Fairly hard	Fairly hard	Fairly hard
4	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Medium	Medium	Medium	Medium	Medium
5	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Soft	Soft	Soft	Soft	Soft
6	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Mobile stiff	Mobile stiff	Mobile stiff	Mobile stiff	Mobile stiff
7	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Mobile soft	Mobile soft	Mobile soft	Mobile soft	Mobile soft
8	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid	Viscous liquid
10	25	Liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Liquid	Liquid	Liquid	Liquid

ence of protease increments, the salt concentration was such as would provide a definite (0.002 or 0.003) molar strength. For the most part, the flour extracts contained a salt concentration of 0.003 M.

In view of the fact that Jørgensen worked with concentrations varying from 150 to more than 300 times the amount that may be used in baking practice, it appeared advisable to secure further data within the

range of practical application according to the method adopted by him. The proteolytic activity of malt syrups and ordinary malt flours is relatively very low and these products do not provide very desirable experimental material. By comparison, Merck's diastase of malt (U. S. P.–IX) is highly proteolytic as are also Taka diastase, raw pineapple juice, and papain. Papain and bromelin are classified as papainases, as is also the proteinase of wheat because each may be activated by the same chemical agents. Later in this paper data are presented which show

TABLE IX

INFLUENCE OF DIALYSIS AND BROMATE ON THE PROTEOLYTIC ACTIVITY OF PAPAIN AND OF RAW PINEAPPLE JUICE [1]

Amount of enzymic preparation added	Temperature	Tests with juice from ripe fruit				Tests with 5% papain extract		
		Controls non-dialyzed	Controls dialyzed	Dialyzed and precipitated material removed	50 c.c. of juice digested with 100 mg. bromate for 2 hours at 35° C. and then dialyzed	Controls non-dialyzed	Controls dialyzed	50 c.c. of extract digested with 100 mg. bromate for 2 hours at 35° C. and then dialyzed
Drops	°C.	Consistency of gelatin after 24 hours digestion at 27° C.						
4	25	Liquid	Viscous liquid	Firm	V. visc. liquid	Viscous liquid	V. visc. liquid	V. visc. liquid
	0	Fairly soft	Hard	Hard	Hard	Hard	Hard	Hard
5	25	Liquid	Liquid	Firm	Viscous liquid	Liquid	Viscous liquid	Viscous liquid
	0	Soft	Hard	Hard	Hard	Hard	Hard	Hard
6	25	Liquid	Liquid	Firm	Viscous liquid	Liquid	Liquid	Viscous liquid
	0	Very soft	Hard	Hard	Hard	Firm	Hard	Hard
8	25	Liquid	Liquid	Firm	Viscous liquid	Liquid	Liquid	Liquid
	0	Mobile	Fairly hard	Hard	Hard	Soft	Firm	Hard
12	25	Liquid	Liquid	Fairly soft	Liquid	Liquid	Liquid	Liquid
	0	Viscous liquid	Soft	Hard	Firm	Mobile	Very soft	Firm
16	25	Liquid	Liquid	Very soft	Liquid	Liquid	Liquid	Liquid
	0	Liquid	V. visc. liquid	Hard	Mobile	Liquid	Viscous liquid	Soft
20	25	Liquid	Liquid	V. visc. liquid	Liquid	Liquid	Liquid	Liquid
	0	Liquid	Viscous liquid	Hard	V. visc. liquid	Liquid	Liquid	Viscous liquid

[1] The proteolytic power of pineapple juice depends considerably upon the degree of ripeness. Juice from unripe fruit is highly proteolytic. When not allowed to ferment, refrigerator stored juice will retain its potency fairly well.

that wheat proteinase responds differently to certain chemicals than do papain and bromelin.

From the results of their studies dealing with the effects of bleaching agents on flour, Balls and Hale (1936) independently reached conclusions in accord with those of Jørgensen. Flohil (1936) arrived at similar conclusions from his study of the effects produced by chemical flour improvers on the proteolytic activity and gas-retaining capacity of fermenting doughs. In this connection attention is directed to the paper

TABLE X

BROMATE ADSORBED BY NORMAL FLOUR BEFORE AND AFTER HEATING TO DESTROY PROTEASE

25 g. samples of flour, each in 100 c.c. of distilled water containing potassium bromate as shown in first column, were allowed to digest in refrigerator over night. They were then centrifuged at high speed and 40 c.c. of the supernatant extract titrated with standard thiosulphate (N/10×1.07).

Bromate dosage	Distilled water controls	Flour extract controls titrated immediately after addition of bromate to flour extract [1]	Normal flour	Heated flour 12 hours at 95° C. and 10 hours at 103° C.
Milligrams	Cubic centimeters standard thiosulfate			
0.5	0.08	0.08	0.05	0.05
1.0	0.15	0.15	0.10	0.10
2.0	0.30	0.30	0.20	0.20
6.0	0.85	0.85	0.65	0.60
10.0	1.32	1.40	1.00	1.05
20.0	2.50	2.60	2.00	2.00
40.0	4.92	5.00	4.00	4.00
80.0	9.98	9.98	8.00	8.00

[1] The presence of starch tends to increase bromate titration due to the tenacity with which the iodine is held by the starch.

TABLE XI

BROMATE ADSORBED BY GUM GLUTEN BEFORE AND AFTER HEATING

Ten gram samples of each of the glutens were digested for 1 hour at 35° C. with 300 c.c. distilled water containing 200 mg. $KBrO_3$. The digest was then centrifuged at high speed and 25 c.c. of supernatant liquid titrated with 0.05 N thiosulfate.

	Cubic centimeters 0.05 N thiosulfate
Bromate control	11.75
American gum gluten as received	11.15
American gum gluten heated in water suspension for 30 minutes at 90° C. to destroy protease	11.15
Hungarian gum gluten as received	11.20
Hungarian gum gluten heated in water suspension for 30 minutes at 90° C. to destroy protease	11.20

by Fisher, Halton and Carter (1937) which presents a vast amount of detailed investigation covering the influence of storage, under varying conditions, on the chemical composition and baking quality of flour. Their data do not indicate that storage reduces the proteoclastic activity of flour.

Mueller (1936) published an interesting study dealing with recent developments in methods of measuring dough quality by physical means. On the basis of experiments designed to show the effect of chemical treatment on the aging and extensibility of flour doughs, Mueller was

unable to support the conclusions of Jørgensen regarding the nature of the action of bromate.

Experimental

Material Used

Gelatin: The gelatin used was a high-grade imported product. Relative proteolytic activity was always measured on 25 c.c. of a 5% solution. A small quantity of thymol was added to prevent bacterial action. Aqueous extracts were made of the enzymic products, unless the product was water soluble. The extracts were centrifuged at high speed to eliminate suspended material as far as possible. The centrifuged extracts and solutions were then added by drops from a 1 c.c Mohr pipette graduated in hundredths. In dropping preparations from the pipette a uniform technic was used throughout the work. The pipette delivered 20 drops of distilled water per cubic centimeter. For any given series of tests made at the same time from the same stock preparation, the size of the drops was quite uniform. Repetition of tests, under like conditions, always gave the same results.

Methods

Dialysis: In the experimental work involving dialysis, nitrocellulose tubing [1] was used. It proved very satisfactory and may be purchased with the desired diameter. Dialyzation was conducted in refrigerator cold distilled water, using large quantities of water to speed up the process. Adjustments were always made to cover any changes in volume which occurred during the course of dialysis.

Bromate determination: Bromate titrations (with sufficient acidity) were made with standard thiosulphate against the iodine liberated by the bromic acid. Titrations were estimated to the second decimal, but these values were "rounded" into first decimal upon averaging two or more separate determinations.

Water soluble nitrogen: Determinations for water soluble nitrogen (Kjeldahl) were made in accordance with the procedure adopted by Jørgensen which has already been described, with the exception that our digestions were vigorously shaken at 15-minute intervals instead of the slow, continuous shaking used by Jørgensen. The effects of varying quantities of different salts in relation to a given dosage of enzymic agent were studied. The use of such a minute quantity of bromate as 0.25 of a milligram per 25 g. of flour comes within the range of commer-

[1] Visking Corporation, Chicago, Illinois.

cial baking practice. The use of approximately 50 mg. of bromate per 25 g. of flour, as employed by Jørgensen is 200 times as great.

Discussion

The data which have been presented in Tables I to XI fail to support the conclusions advanced by Jørgensen and certain other investigators who hold to the same opinion, namely, that the benefits commonly derived from the use of bromate in flour doughs result directly from its inhibitive action on the proteinase contained in the flour.

Against papain and bromelin (as the latter exists in raw pineapple juice) bromate does exert an inhibitory action, but such does not appear to be the case for the proteinase of flour. Neither does bromate act unfavorably toward the protease of Taka diastase, or toward pepsin and trypsin. This would seem to raise anew the question as to whether or not the proteinases of wheat and barley are true papainases as now classified.

The decrease in water soluble nitrogen (Tables I and II) which Jørgensen attributed to proteinase inhibition may be explainable on other grounds. The surprising response of colloidal gels to very minute traces of certain electrolytes is a well established fact. The "toughening" action of bromate on gluten is well known. The excessive amounts of bromate used by Jørgensen may have prevented certain cleavage products of the gluten from passing into solution because of a purely colloidal condition.

Although it is a foreign protein, gelatin has been used for many years as a convenient substrate on which to measure relative proteolytic power, and much of the information now available has been gained therefrom. It would appear that any agent which does not adversely affect proteolysis when gelatin is the substrate may reasonably be expected to behave in like manner when gluten is the substrate. It is realized, however, that such a position is not altogether tenable. Among the several proteases studied by this means, papain and bromelin were the only two proteases inhibited on gelatin by the action of bromate. Pepsin, trypsin and the proteinases of malt and Taka diastases were not repressed.

These results caused us to seek other methods of attack. It was thought that digestion with bromate and subsequent dialysis might lead to more definite information. Consequently, water extracts and solutions of soluble enzymic products were prepared and digested with relatively excessive quantities of bromate prior to dialysis. The dialyzed products were then tested with a view to determining to what extent proteolytic activity had been impaired. Both papain and bromelin were weakened by contact with the bromate, but malt proteinase and the pro-

teinase of Taka diastase were not impaired. The pH of the enzyme preparations was approximately the same as that of bread dough—if anything more strongly on the acid side. The conditions, therefore, were just as favorable for bromate attack as would be the case in bread dough. The results obtained have been recorded in Tables VIII and IX.

The effect of dialysis on the proteolytic activity of pineapple juice should be noted. Upon removal of the dialyzable substances contained in the juice, an appreciable quantity of coagulated matter appears. When this coagulum is centrifuged out, it removes practically all of the bromelin. Otherwise, the potency of the juice is not materially lowered by dialysis.

The results of experiments designed to show approximately to what extent bromate may be adsorbed by flour and by commercial gum gluten are shown in Tables X and XI. If proteinase is held firmly by the gluten complex as is now believed, the commercial processes involved in the manufacture of gum gluten would not be expected to remove or destroy the adsorbed proteinase. We believe this may be particularly true of the gum gluten received from Budapest, Hungary. When wetted and given a little kneading, this gluten did not differ in "feel" from that of gluten freshly prepared by hand. The other sample of domestic manufacture behaved more like a partially denatured product. It will be observed that each gluten adsorbed bromate to some extent, and that complete destruction of adsorbed proteinase did not affect the adsorption. It will also be observed that flour itself shows a similar adsorption which is to be attributed, presumably, to the gluten complex.

Summary

Experimenal data (Tables I and II) have been presented which show the effects of varying quantities of bromate on the activity of malt proteinase, papain, bromelin, pepsin, trypsin, and the protease of Taka diastase as determined by water soluble nitrogen. When applied in amounts suitable for commercial use in the bake shop, no appreciable inhibitive action was apparent. From Table II it will be observed that the presence of vitamin C produced an increase in soluble nitrogen, except for trypsin. Sodium bisulphite likewise increased the values for soluble nitrogen. It might thus be regarded as an activating agent, but other data indicate that such is not the case.

Bromate did not adversely affect the proteolytic power of malt, pepsin, trypsin, or Taka diastase when these products were allowed to act on gelatin, but it did repress the activity of bromelin and papain when present in relatively large quantities in proportion to enzyme (Tables III to VII). The effects of salts other than bromate are also

shown. It will be noted that the behavior of sodium metavanadate is quite similar to that of bromate and that sodium bisulphite tends to repress rather than activate. This is contrary to certain statements which have appeared in the literature.

Digestion with bromate for two hours at 35° C. and subsequent dialysis of the bromate indicated no destructive action by the bromate on the proteases of malt and Taka diastase, but bromelin and papain were adversely affected. Pepsin and trypsin were not studied from this angle.

The data contained in Tables X and XI indicate to what extent bromate may be adsorbed by the gluten complex before and after the destruction of proteinase.

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THE DETERMINATION OF SUCROSE IN FLOUR

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(Read at the Annual Meeting, May 1937)

Flours vary widely in sucrose content as has beeen shown by Blish, Sandstedt, and Astleford (1932). Sandstedt (1934) reports sucrose values on a series of flours ranging from 84 mg. to 225 mg. per 10 g. of flour. These differences in sucrose values are great enough to be of considerable influence on the gassing powers of these flours and accordingly it often becomes necessary to supplement the knowledge gained from the maltose value by a determination of sucrose. It would greatly facilitate the determination of sucrose if it could be determined directly from the flour extract which has been made for the maltose determination. Sucrose hydrolyzes 976 times as rapidly as maltose (Gortner, 1929) so it should be possible to hydrolyze completely the sucrose in such a flour extract with no appreciable hydrolysis of the maltose. Since sulfuric acid is added to the flour extract for clarification, it would seem that this could serve also for sucrose hydrolysis.

In order to check these possibilities, solutions of pure sucrose, maltose, and mixtures of sucrose and maltose corresponding to the concentrations of these sugars in flour extracts were used. These solutions were treated with buffer (pH 4.7), sulfuric acid, and sodium tungstate, as is usual in the preparation of a flour extract for maltose determination. Five cubic centimeter aliquots of these sugar solutions were placed in test tubes and submerged in a boiling water bath. It was found that the sucrose was completely hydrolyzed in 13 minutes and that 13 minutes' boiling had no appreciable hydrolytic effect on the maltose.

Based on these findings the following method was developed: 5 c.c. of the filtered, clarified flour extract (made according to the specifications for maltose determination) are pipetted into an eight-inch test tube and immersed in a vigorously boiling water bath. After 13 minutes' boiling the test tube and contents are cooled under running water and 10 c.c. of alkaline N/10 ferricyanide are added. The reduction of the ferricyanide and the subsequent titration are carried out as in the determination of maltose by the ferricyanide method. The ferricyanide reduced by the maltose in the flour is subtracted from the value obtained after hydrolysis and the sucrose represented by the difference is obtained from the Ferricyanide Sucrose Table.

0.10 N Ferricyanide Sucrose Conversion Table

0.10 N Ferricyanide reduced	Sucrose per 10 g. flour	0.10 N Ferricyanide reduced	Sucrose per 10 g. flour	0.10 N Ferricyanide reduced	Sucrose per 10 g. flour
C.c.	*Mg.*	*C.c.*	*Mg.*	*C.c.*	*Mg.*
0.10	5	3.00	143	5.90	280
0.20	10	3.10	148	6.00	285
0.30	15	3.20	152	6.10	290
0.40	19	3.30	157	6.20	294
0.50	24	3.40	161	6.30	299
0.60	29	3.50	166	6.40	304
0.70	34	3.60	171	6.50	309
0.80	38	3.70	176	6.60	313
0.90	43	3.80	181	6.70	318
1.00	48	3.90	185	6.80	323
1.10	52	4.00	190	6.90	328
1.20	57	4.10	195	7.00	333
1.30	62	4.20	200	7.10	337
1.40	67	4.30	204	7.20	342
1.50	71	4.40	209	7.30	347
1.60	76	4.50	214	7.40	352
1.70	81	4.60	218	7.50	357
1.80	86	4.70	223	7.60	362
1.90	91	4.80	228	7.70	367
2.00	95	4.90	233	7.80	372
2.10	100	5.00	238	7.90	377
2.20	104	5.10	242	8.00	382
2.30	109	5.20	247	8.10	387
2.40	114	5.30	251	8.20	392
2.50	119	5.40	256	8.30	397
2.60	123	5.50	261	8.40	402
2.70	128	5.60	266	8.50	407
2.80	133	5.70	270	—	—
2.90	138	5.80	275	—	—

Note that the above sucrose method calls for the use of the modified ferricyanide method using N/10 reagents. If the original N/20 ferricyanide method is used, only 3 c.c. of the flour extract are taken for hydrolysis. After hydrolysis these are diluted to 5 c.c. with water and the procedure continued as above.

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A COMPARISON OF 1X, 2XX, AND 4XXXX TIN PANS IN EXPERIMENTAL BAKING [1]

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(Read at the Annual Meeting, May 1937)

Introduction

The standard A.A.C.C. baking test requires the use of official A.A.C.C. baking pans, either of the tall or low form, and constructed from 2XX tinned metal. The query as to the value of using any specific weight tin in experimental baking has never been answered. Various laboratories have used or are using, with apparent satisfaction, baking tins which are of heavier or lighter gauge tin than the regular 2XX pan. Davis, Leatherock, and Putnam [2] state that when light and heavy gauge black metal pans were used, the heavy gauge decreased loaf volume in the tall-form pan 10% to 12% and the loaf type was changed. This fact was thought to be very significant and further studies with reference to tinned metal should prove interesting and valuable.

The commercial production of pan bread requires a baking pan which will give long service with constant use and be satisfactory for all existing conditions in the shop. 4XXXX tin metal is probably most universally used in commercial practice. Many laboratories hoping to maintain testing conditions as close as possible to commercial conditions prefer to use 4XXXX tin pans. This study was undertaken to determine the relative merits of different gauge tinned metal in experimental baking pans.

Experimental

Pans of the A.A.C.C. tall form dimension and of a slightly modified A.A.C.C. low form dimension constructed of 1X, 2XX and 4XXXX tinned metal were used in this experiment. The tall form tins weighed 128 g., 141 g., and 180 g., respectively, and the low form tins weighed 106 g., 125 g., and 145 g., respectively.

The three flours used were selected with the object of testing the pans in distinctly different loaf volume ranges, and with different type doughs.

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[1] Subcommittee report, 1936–37 Committee on Standardization of Laboratory Baking.

[2] Davis, C. F., Leatherock, L. E., and Putnam, H. W. Experimental baking pans. Cereal Chem. **13**: 113–118 (1936).

The straight dough formula used on flours 1A and 3B was the A.A.C.C. basic formula modified by increasing the sugar to 4% and adding 2% shortening. All manipulations were essentially those of the tentative A.A.C.C. baking test procedure. The sponge formula used on flour 2A consisted of 2% salt, 4% sugar, 2% yeast, 2% shortening and .35% yeast food. All doughs were proofed to a constant height with an average time of about 50 minutes.

Each dough mix was based on 300 g. of flour and was divided into three doughs of 160 g. each. All bakes were made in a series of twelve loaves per day on successive days. In this way duplicate results were obtained on each pan each day, and the error includes the day to day variability in baking results.

In Table I is recorded a loaf volume summary of the results of eight bakings in each pan. The mean loaf volumes and variability of results are not significantly different for the different pan weights. Loaf characteristics such as crust color, break and shred, and grain and texture were likewise apparently unaffected.

TABLE I

A Comparison of 1X, 2XX and 4XXXX Tin Metal in Tall and Low Form Experimental Baking Pans

Flour	Method	Weight of pan	Low form A.A.C.C. pan (modified)			Tall form A.A.C.C. pan		
			Number of loaves	Mean volume	C.V.[1]	Number of loaves	Mean volume	C.V.[1]
				C.c.			*C.c.*	
1-A	Straight dough	1X	8	667	2.60	8	663	2.10
		2XX	8	664	2.30	8	654	2.30
		4XXXX	8	666	1.60	8	658	1.80
2-A	Sponge dough	1X	8	748	1.82	8	755	1.23
		2XX	8	755	1.10	8	748	1.89
		4XXXX	8	749	.93	8	746	1.18
3-B	Straight dough	1X	8	672	2.30	8	632	2.22
		2XX	7	662	1.72	7	624	2.21
		4XXXX	8	674	1.47	7	627	2.03

[1] Coefficient of variation.

Conclusions

Loaf volume, loaf volume variability and loaf characteristics are not definitely affected by 1X, 2XX or 4XXXX tin metal in the construction of experimental baking pans. The service required of small experimental pans should not necessitate the use of 4XXXX tin for such pans.